Investigation of potato cyst nematode control

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PREFACE

In the writing and collating of this document, representatives from key sectors of the potato industry and agricultural research were consulted. A pre-submission committee was convened in October 2002 to discuss the direction and content of a proposal in response to the Defra call and the format and agenda of an open forum. In addition, a mainly industry led steering committee was formed to review the document near its completion. We greatly appreciate the involvement and positive contributions that the members of the two committees made to the final document. They are as follows: Graeme Byers (Higgins Ltd), Tudor Dawkins (Du Pont), Mike Harrison (Farmacy), David Nelson (Branston Potatoes Ltd), Jon Pickup (SASA), and Mike Storey (BPC).

We would like to thank all the representatives from the industry, and from the donor and scientific communities who attended the open forum. In particular, those who made presentations or contributed to the consequent discussions listed in Appendix 11.3, all of which contributed to the assimilation of the recommendations included in this document. The forum was attended by more than sixty delegates and provided valuable information for generating the recommendations for research in the review.

We would also like to acknowledge the technical editing and contribution made by Mark Phillips (SCRI) to those sections covering aspects of plant breeding, including genetic engineering.

The authors would like to thank Dr Helen Jacobs for her considerable help in locating and assimilating references and for the final preparation of this document. She also acted as coordinator for the organisation and running of the successful two day open forum. Her scientific knowledge of the management of potato cyst nematodes and her editorial skills are much valued.

SUMMARY

The UK potato crop is important in maintaining the economic viability of more than 500,000 ha of productive farm land and the potato industry employs more than 30,000 people. Potato production, therefore, is vital for rural livelihoods and important for food security. Potato cyst nematodes (PCN) remain the most important constraint to potato production in the UK and elsewhere in Europe. Although some other European countries, including the accession states, may have sufficient potato land to maintain long rotations, PCN will inevitably spread to these areas and sustainable production will require the development of suitable resistant cultivars and alternatives to current nematicides. Research funded by the EU has been instrumental in the development of collaborative projects between UK nematologists and those elsewhere in Europe, including the main centres in the Universities of Wageningen in The Netherlands, Ghent in Belgium and Coimbra in Portugal. Much of this research has been targeted at *G. rostochiensis*, which remains an important pest in mainland Europe, and at understanding the molecular interactions between PCN and its host, which is a demanding area of research and requires long term investment before new management products are likely to become available. A range of R & D projects to provide improved control measures for PCN are in progress, including the production of GM cultivars with resistance to PCN, which are evaluated in this report. Within this background, the report reviews current research and makes recommendations for the future directions of research effort. This is done in the understanding that:

- research and development from both academic and industrial sources has led to the successful control of *G. rostochiensis*
- *Globodera pallida* is spreading and sustainable IPM strategies must continue to be developed for all situations to reduce dependence on current nematicides and the environmental impacts of producing potatoes on PCN infested land
- the area of economically viable, PCN-free land for growing potatoes is very limited
- continued and increased support by the government of research into an important and pernicious pest demonstrates a confidence in the future of the industry. Those growers remaining are predominantly those who have adapted to a technically demanding industry and are motivated towards adopting new methods and strategies
- nematicides are expensive to use and growers are receptive to changed management methods that reduce dependence on such chemicals
- reducing funding into research on PCN will threaten the continuation of plant parasitic nematology in the UK. However, better co-ordination between, and collaboration within, the UK research and donor community is essential to maximise benefits to funding agencies and the industry

- research on the genomics of PCN will provide new options for their management but will require a co-ordinated effort within the European nematological research community in which Defra could play a key role

The control of PCN requires relatively large dosages of pesticides that were developed more than 30 years ago. There is a need to develop strategies that reduce their use and that employ more environmentally benign methods. The development of such strategies will continue to require the active support of Defra.

1. BIOLOGY OF POTATO CYST NEMATODES

Potato cyst nematodes (PCN) are sedentary, endoparasitic nematode pests. Within a typical life cycle there is a moult within the egg to produce the infective, second-stage juvenile. These juveniles are the resting stage in the nematode's life cycle, and the majority only emerge from the egg after receiving stimulation from a growing host crop. They can remain dormant within cysts in soil for up to 25 years before they emerge from eggs, migrate through soil and invade a host root in the zone of elongation behind the root tip. Long rotations with non-host crops may be essential to reduce heavy infestations in soil. The second-stage juvenile must establish a feeding site (syncytium), a transfer cell, which supports the rapid transfer of photosynthates from the stele to the developing nematode. At this stage the nematode begins to swell and is sedentary. Failure to produce a fully developed feeding cell results in the death of the female nematode and is the basis of the resistant reaction. There are a further three moults before the adult stage is reached. The female is so enlarged that she ruptures the root cortex and is exposed in the rhizosphere, where she continues to feed from the syncytium during egg production. Adult males regain their worm-like shape and migrate from the roots to fertilise the females. Abiotic and biotic factors that influence the size of the feeding cell may affect the sex of the nematode; females require much more food to produce eggs and to maximise their fecundity compared to the resources required for males to reach adulthood. Thus, in conditions of stress, female juveniles may fail to complete their development and may change sex so that populations are dominated by male nematodes. Such feed-back mechanisms help regulate PCN abundance. There is one generation in a growing season but their large potential reproductive rate (more than ×100) enables populations in soil to build up to levels of 10^3, and in roots to densities of 10^4, individuals per gram. Such infestations are not unusual and are very difficult to manage. In theory, control agents must be 98% efficient to prevent population increase. Invasion of roots by second-stage juveniles alters the morphology of the potato root system and is the principal cause of yield loss but measures that affect numbers of females are likely to have the greatest effect on population control and long-term infestation levels in soil.

Like all plant parasitic nematodes, PCN are obligate parasites and must feed on plant hosts to complete their life cycle. Hence, they must enter the rhizosphere to reach their host and develop there during female maturation, where they may interact with fungi and bacteria. In the rhizosphere, there may be >60 times more bacteria and >12 times more fungi than in the bulk soil. PCN have a narrow host range amongst the Solanaceae and are likely to depend on signals from their host that affect root location and survival. For example, the hatch of eggs is greatly stimulated by exudates from the roots of host plants. Such interactions with the host and microbial community provide opportunities for intervention and new control strategies.

In 1973, PCN were recognised as two sibling species, *Globodera rostochiensis* and *Globodera pallida*. The latter species is now dominant in potato land in England and Wales and is proving much more difficult to control than *G. rostochiensis* because:

Populations are more genetically diverse and only partial resistance sources are available (see Section 4)

Eggs often hatch at a slower rate and the second-stage juveniles have greater lipid reserves, which enable them to remain viable in soil for longer. Control by nematicides is, therefore, less effective (see Section 8)
Population decline rates between potato crops are less and longer crop rotations are needed to reduce populations to non-damaging levels (see Section 7)

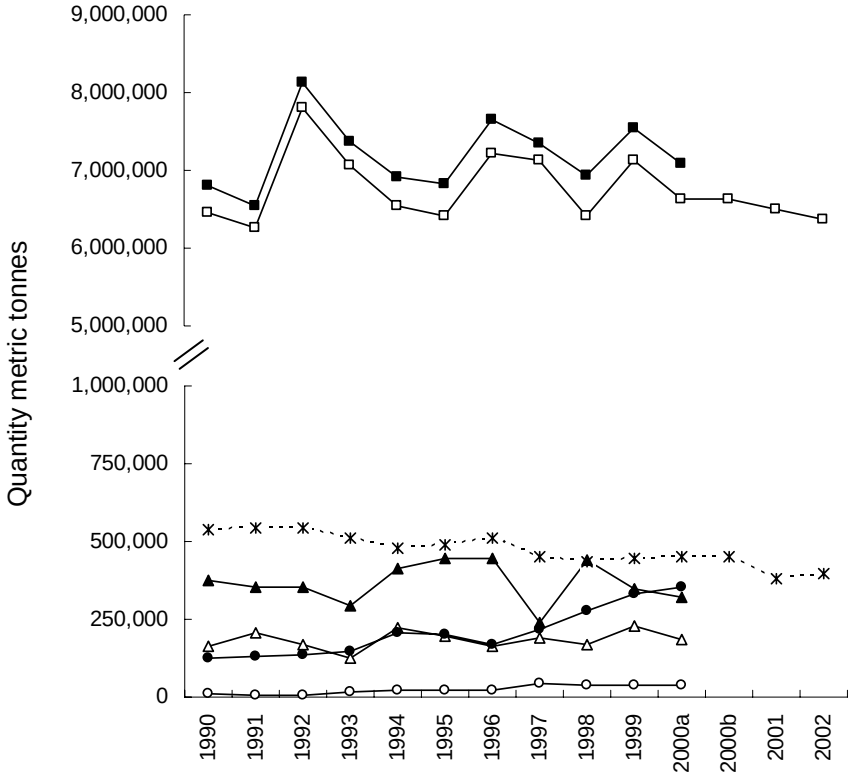
As a consequence, PCN have become the major pest constraint to potato production in the UK. This study critically assesses the options available to growers for the short-term and longer term (>5 years) research needs to provide more acceptable methods for their management.

2. STATUS OF THE POTATO CROP IN THE UK AND EFFECT OF THE PCN EPIDEMIC

2.1 The importance of the potato crop to UK agriculture

To assess value of research into the control of potato cyst nematodes (PCN), the importance of the potato crop to UK agriculture as a whole, and to rural communities in potato growing areas, should be considered, and not just the profitability of the crop on a per hectare basis. The Defra 2003 Agricultural and Horticultural Census [1] showed that there were some 148,000 ha of potatoes grown in the UK, which represented approximately 3.7% of all arable crops. However, as the average rotation length for potatoes is five years and the total land area for all horticultural and agricultural crops is 4.6 million ha (not including fallow and set-aside), 16.1% of farm land is used by the UK potato crop. There is an annual production of 6 million tonnes, of which 70% is stored, allowing a controlled supply to the market place. The ex-farm value is £700M, which is between a quarter and a third of the value of the cereal crop, which has a retail value of £3.5 billion (BPC, PCN Review Open Forum [2]). This compares with a value of approximately £250M for sugar beet grown on 166,000 ha.

Figure 2.1 Production, import and export of potatoes in the UK. (■) UK potato production, (□) total UK potato consumption, (Δ) imports, (▲) exports, (○) frozen imports, (●) frozen exports, and (x) seed.



Source: Food and Agriculture Organization of the United Nations [3]

More than 100kg/person/annum of potatoes are purchased in the UK, of which 50% is fresh with a value of more than £750M. Potatoes make up 75% of the expenditure on carbohydrates in the UK, with pasta and rice making the remaining 25% [2]; consumption of frozen, processed potato foodstuffs continues to increase (Figure 2.1). The increase in the value per unit makes transport costs more affordable and, therefore, imports more attractive to the supermarket chains. Currently, however, this latter is only a small part of the market and the value is low in comparison to the overall UK consumption, but the trend is clear.

There are also various niche markets and, in particular, cultivars that are only supplied by UK growers (D. Nelson, pers. comm.). There is also the attraction of local sourcing, which is used by some supermarkets to promote potato sales as well as allowing them a more direct influence on how the crop is grown.

Some 30,000 people are directly employed by the potato industry [2], including those in the packing and processing units. In areas such as those around the Wash and in East Anglia, potato production and packing for the supermarkets is a vital part of the local economy. The sale of tied cottages and the rapid increase in house prices, in villages where demand from the commuter market has accelerated the property values, agricultural workers and their associated communities depend on the local field vegetable and potato industries, which pay above average wages.

2.2 The economic importance of the potato crop in maintaining farm viability

The influence of potato growing on the economic viability of arable farming is apparent when the gross margins of the main arable crops are compared.

Table 2.1 Gross margins of the main arable crops

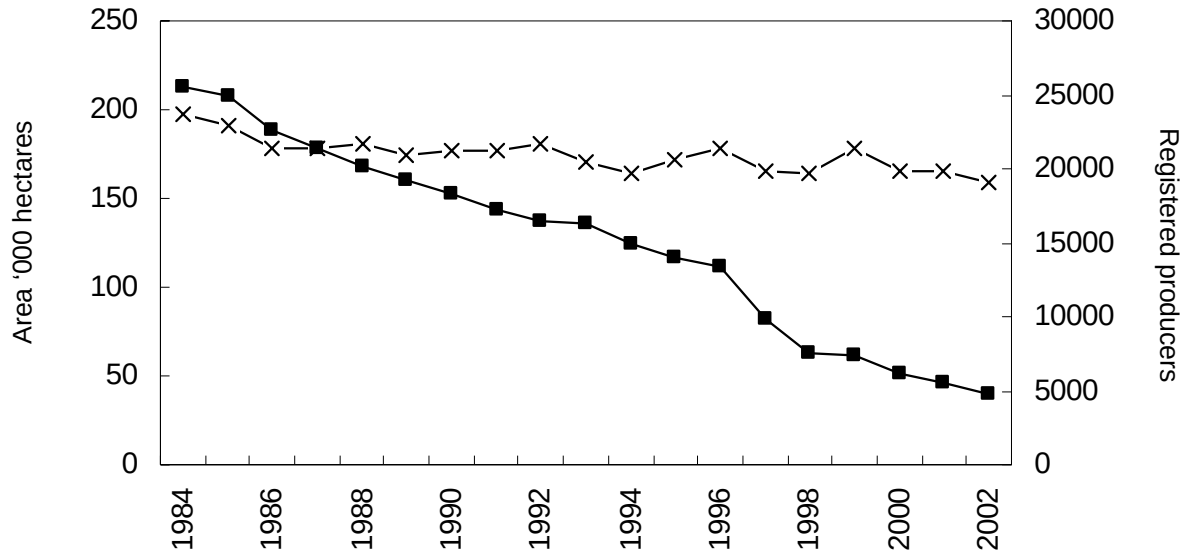
Crops	Yield t/ha	Price £/t	Gross Margin £/ha
Winter wheat (feed)	8.5	70	586
Winter wheat (milling)	7.8	90	678
Winter barley (malting)	6.0	80	533
Oilseed rape	3.4	155	517
Sugar beet	54.0	31	1,067
Onions	40.0	100	1,047
Potatoes (early)	**25.0**	**135**	**1,722**
Potatoes (main) 2002 Oct	**40.0**	**66**	**515**
Potatoes (main) 2003 Oct	**40.0**	**110**	**2275**
Potatoes (main) 5 year average	**40.0**	**100**	**1,875**

(Adjusted estimates taken from various sources [4, 5, 6])

Taking into account a fixed cost of between £600/ha and £825/ha, depending on the size of the farming unit, a successful potato crop can make it a viable enterprise. The margins for the cereals and rape include the £238/ha of area payments. If 'decoupling' becomes part of the EU policy, the margins shown could lead to some cereal crops

being no more than break crops. The marked variation in price per tonne of potatoes between years is characteristic of this unsupported crop and can lead to obvious budgeting difficulties. However, although the number of growers involved in potato production has fallen, the area grown has only marginally decreased in recent years (Figure 2.2).

Figure 2.2 Number of UK registered potato growers (■) and area of potatoes grown (x) between 1984 and 2002.



Source: Defra and BPC (Potato Statistics in Great Britain 1998-2002)

2.3 The economic cost of PCN in the UK

The economic cost of PCN to the UK potato growing industry was estimated at more than £43M in 1998 [7] based on lost yield alone. This figure does not include the indirect costs such as increased fertiliser and irrigation use to compensate for poor crop performance due to PCN infestations (identified or otherwise), and the cost of research both publicly and commercially funded. A survey in 1999 [8] found that PCN was present in 64% of potato fields sampled in England and Wales. The numbers found were very variable and, due to the patchiness of PCN infestations at field scale, these results are not representative of even approximate levels across particular fields but they do give an indication of the increasing incidence of *G. pallida* in potato land when compared to previous surveys [9].

The direct cost of managing a field suspected of containing PCN is the initial expense of sampling and then the possible cost of the nematicides, which may be as high as >£900/ha if both the fumigant Telone™ (1,3-dichloropropene) and a granular nematicide are used. Due to the intrinsic difficulty and expense of accurately sampling a field for PCN, the current recommendation by many agronomists (J. Blaylock, pers. comm.; M. Harrison, pers. comm.) and researchers is that a granular nematicide is applied to all of a field if any cysts are found, regardless of how few or of the viability of the eggs inside. In contrast, due to the cost of its application, 1,3-dichloropropene has been recommended as a treatment only above a threshold of 20

eggs/g of soil but, in many areas, this has recently been reduced to 17 eggs/g of soil (M. Harrison, pers. comm.). This is partly because granular nematicides can vary in their efficacy to control PCN and, with an investment of *c.* £3,000/ha, growers may be prepared to invest further to reduce the risk of yield loss. However, to some degree, these thresholds are based on experience and the decision should consider additional factors, such as previous cropping history, soil type, cultivar and sampling results.

On many farms the potato crop is the prime cash crop and a high cost for a unit is the extension of rotation required for PCN control, which greatly reduces the overall profitability of those fields or areas contaminated. Renting land specifically for potato growing is an option for some growers but is only cost-effective if the land is local and the agricultural unit has the capacity to crop an increased area of the other crops in the rotation that replace the original potato area (R. Howard, pers. comm). Smaller units are less likely to have the capacity for expansion as labour can be the largest overhead, and this will increase disproportionately with the increase in overtime that would be required [10]. There is also a practical and economic threshold for the increased use of machinery. The decisions to employ another worker or purchase larger machinery are strategic ones and there must be some confidence in the future of the potato industry before such overheads are increased. To this extent, the continued development of sustainable approaches and methods for the control of PCN will favour the smaller producer more than the larger.

On a larger scale, the moving of the potato crop to uninfested areas has two main drawbacks. The traditional ware producing regions already exploit the most productive soil types and, in many areas, have access to established water systems and infrastructure for irrigation [11]. Technical expertise and other sections of the supply chain such as packing houses are also localised. The other important drawback is that PCN would eventually reach new areas, probably through transport on farm machinery or vehicles, negating any short-term benefits.

It is likely that PCN will continue to spread across Europe, where the pest is already present in 35 countries [12], and switching sourcing to PCN-free areas outside the UK will only postpone the problem and, in the process, greatly reduce the likelihood of good traceability and the influence of the consumer. The increased transport costs that would be involved are not sustainable in the long term. Although potato production in Eastern Europe currently experiences lower overheads, the trend is towards parity with Western Europe as economies develop.

2.4 The use of nematicides for the control of PCN and the influence of the supermarkets

In 2002, 27% [13] of the area of the potato crop was treated with nematicides and, allowing for areas treated with both fumigant and granular nematicide, *c.* 30,000 ha are treated annually, with an approximate cost of £10M. In 2002, an average of 8.18 kg a.s./ha of nematicides were applied, more than four times the total amount of pesticides applied to most other crops.

Pesticide residues in fresh produce are understandably of greatest concern to the consumer [14] and this has prompted some supermarket chains to limit the amounts of nematicide that may be used by individual growers and, if necessary, to source

produce outside the UK. This is a tactical rather than strategic approach and has already led to problems with sustaining consistency and quality in supply. However, although nematicides are an unfortunate necessity in maintaining the economic viability of some potato growing areas, supermarkets, through the promotion of their own grower protocols, are helping to reduce the quantity of nematicides used. An increase in the public awareness of nematicide use, brought about by the introduction of the Pesticide Tax or the discovery of residues, could damage the image of the potato crop as a healthy food [14].

2.5 Research and PCN

Research in the UK has made important advances in the understanding and control of PCN, including the identification of the two species that helped to explain the poor control by nematicides, crop rotation and resistant cultivars. The basic biology and behaviour of the two PCN species are now better understood, and this has led to the development of more targeted control methods.

The research into the sustainable control of PCN is necessarily broad as it is evident that, for *G. pallida*, no single technique will be sufficient. An integration of different methods and approaches will provide the flexibility needed to control this pest in a range of different circumstances, including organic production. Although PCN is the focus of much of the nematological research in the UK, advances in understanding the biology of the pest and the methods for its control subsequently developed extend not only to other nematode species but to other pest problems.

The level of plant parasitic nematode expertise and knowledge in the UK is recognised worldwide and the exchange of information between international research centres greatly increases the productivity and success of research here. Although agricultural situations overseas can be very different, there are many aspects that are transferable to the UK with local adaptation. However, it is of obvious importance that established nematode research groups be situated where they can exploit research and techniques used in other fields, and that they have good access to the UK agricultural industry. As PCN is currently the most important nematode pest species in the UK, funding research into its biology and control etc. is the major source of finance for UK nematology research groups. Any further reduction in funding in this area may well reduce the number of plant nematologists in the UK to below a sustainable level. In addition to PCN and other free-living nematodes currently recognised as economically damaging pests on crops in the UK, there is the very real threat of climate change. What are occasional observations of non-UK pest species being found in glasshouses may well lead to more serious pest problems on outdoor crops [15, 16, 17].

2.6 Research funding on PCN in the UK

There is a considerable interest by the potato industry in research on PCN and its control but agriculture is experiencing a severe downturn in profitability and, unless the research is very near-market, any support for research from the industry is mainly in-kind in nature. Although such support is essential, financial support is obviously also necessary. An important source of continued financial support from the potato growers has been through the British Potato Council (BPC). However, BPC's revenue

comes from a levy on the crop, resulting in a restricted budget that must cover all aspects of potato production, not just the problems caused by PCN.

Current research would suggest that nematicides should be applied to all land infested with PCN to help prevent *G. pallida* from reaching economically damaging levels. This would mean increasing current nematicide application from 25% to 64% of potato land. Provision of more sustainable management options for growers will require an increased commitment of funds for a prolonged period. This would call for better coordination of research effort and increased commitment from the research agencies in the UK.

2.7 Recommendations

- *Defra establish a research and development committee to coordinate longer term objectives and funding for research on PCN, with representatives from the funding agencies, research teams and industry.*
- *Increased funding for research into the biology and control of* Globodera pallida*.*
- *Future research projects on PCN should include aspects of work on other UK plant parasitic nematodes.*

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3. THE PCN EPIDEMIC

3.1 The introduction of PCN to Europe and the UK

Potato cyst nematodes are thought to have co-evolved with their plant hosts (potatoes and other members of the Solanaceae) at altitudes of 2000 m or more in Andean regions of South America [1]. Potatoes were introduced into Europe in about 1570 [2] but PCN were probably introduced much later, on tubers taken to Europe from South America as part of a search for late blight resistance in response to the blight epidemics of the 1840s [3]. The areas from which potato tubers were sourced in South America would have influenced the species of PCN that they carried. Further introductions were probably made over a period of years, although molecular analyses of the relatedness of European populations of PCN suggest that there have been relatively few introductions [4]. Some of the introductions may have been via routes other than as contaminants of potato tubers, such as in guano shipments transported in old, PCN-contaminated potato sacks [5].

3.2 The emergence of PCN as a threat to potato production

The first records of PCN associated with damage to a potato crop in Europe date from about 1881 in Germany and the evidence suggests that PCN were present in the UK by 1900 [3]. The time taken for PCN damage to appear in crops obviously depends on the frequency with which potatoes are grown but damage usually occurs within five or six crops after the date of introduction, or about 20 years when potatoes are grown on 4-year rotations [6, 7]. Depending on the ability of the cultivar being grown to tolerate PCN attack, and the population density of the nematodes, crop losses caused by PCN can be up to 100%.

3.3 The current incidence of PCN

Advisory sampling for the presence of PCN in land intended for potato production has provided some estimate of the incidence of PCN and, in the five years up to 1986, 62% of samples submitted to ADAS laboratories in Cambridge, Leeds and Newcastle were PCN-infested [8]. However, this value is probably biased because sampling would tend to be concentrated on farms known to be infested. A subjective survey by the Potato Marketing Board in 1992 estimated that 42% of potato fields were infested [9], but analysis of a sub-set of samples taken only from ware potato fields in 1994/5 indicated that 67% were PCN-infested [9]. The most accurate figure for incidence currently available comes from a structured and statistically unbiased survey of potato land in England and Wales, in which PCN were found in 64% of 484 sites sampled in 1997/8 [10].

3.4 Changes in the incidence of the two species of PCN

Before the first potato cultivars with resistance to PCN became available, most importantly Maris Piper, *Globodera rostochiensis* was the more prevalent of the two species of PCN in Scotland, Northern Ireland, and the major potato producing areas of south Lincolnshire and East Anglia, whilst *G. pallida* was the more prevalent in the East Midlands and northern England. The early cultivars with PCN resistance carried the *H1* gene, effective only against certain races of *G. rostochiensis* but including all

known UK populations of this species. No cultivar carrying only the *H1* gene (e.g. Maris Piper, Cara, etc.) has resistance to *G. pallida* and use of such cultivars can decrease population densities of *G. rostochiensis* by up to 80% whilst, at the same time, allowing *G. pallida* to reproduce unhindered. Cowton (1983) [11] confirmed experimentally the earlier predictions that the use of *H1* cultivars would lead to the predominance of the harder to control *G. pallida* and, by 1986, there were indications that a switch from one species to the other was already happening in East Anglia [8]. By 1996, *G. pallida* had become the dominant species in East Anglia [9] and it is now the dominant species throughout England and Wales. In two separate surveys, the presence of *G. pallida* has been confirmed in 90% [12] and 92% [10] of samples containing PCN collected from potato fields. Market forces have caused potato producers to grow a predominance of cultivars with resistance only to *G. rostochiensis*, some 43-45% of all plantings [10], and this is the main cause of the switch to *G. pallida* that has occurred (see Table 3.1 for a list of cultivars, areas grown and their resistance status).

Table 3.1 Potato cultivars grown on over 2000 ha in 1999 (BPC) and their resistance status towards PCN

Cultivar	Area grown in 1999 (ha)	% of total ware area	Resistance status		
			G. rostochiensis	*G. pallida*	Susceptible
Maris Piper	30 998	21.2	r*		
Estima	14 432	9.9			✓
Cara	8 666	5.9	r		
Saturna	7 292	5.0	r		
Pentland Dell	6 350	4.3			✓
Nadine	6 326	4.3	p.r.*	p.r.	
Hermes	6 071	4.1			✓
Désirée	5 969	4.1			✓
Marfona	5 135	3.5			✓
Lady Rosetta	4 824	3.3	r		
Maris Peer	3 561	2.3			✓
Première	3 295	2.2	r		
Maris Bard	3 255	2.2			✓
Wilja	2 889	2.0			✓
King Edward	2 845	1.9			✓
Russet Burbank	2 770	1.9			✓
Santé	2 112	1.4	r	p.r.	
Total	116 790	77.3	43.3	5.7	36.2

* r = fully resistant; p.r. = partially resistant

3.5 What the future holds

Future changes in the incidence and distribution of PCN will depend upon the effectiveness of control measures that are imposed. If we carry on as we are, then, quite simply, *G. pallida* will eventually completely replace *G. rostochiensis* and the management of PCN will become more difficult than ever. At present, most farmers integrate the use of nematicides with rotation and a few also use cultivars with partial

resistance to *G. pallida*. To analyse the future impact of such strategies requires the facility to model the interactions between control methods. Great progress has been made in recent years in this respect and Trudgill *et al.* (2003) [12] have shown that control of *G. pallida* by rotation alone may require rotations of up to 18 years. Introduction of a granular nematicide would probably allow rotation lengths to be decreased but lengths of up to 12 years would probably still be required. Although cultivars resistant to *G. pallida* have only partial resistance, management systems that rely on these would probably require a minimum rotation length of 9 years, providing 75% control could be maintained with each crop. As field populations of *G. pallida* tend to show increased virulence towards a particular partially resistant cultivar each time that it is grown, growers would need a choice of different cultivars to allow effectiveness to be maintained. Currently, there is insufficient choice of partially resistant cultivars for growers to meet the requirements of supermarkets. Indeed, only *c.* 8% of the potato area is planted with partially resistant cultivars and much of this (*c.* 40%) is on land not known to be infested with *G. pallida* [10]. Thus, with a current average rotation length of 5.7 years [10], the *G. pallida* problem is set to increase. We need to take action now to protect uninfested land from possible contamination, and to make greater use of integrated control policies on infested land, particularly the wider deployment of partially resistant cultivars but also the use of alternative control measures such as trap cropping. With appropriate tools and appropriate advice, the problem can be managed in a sustainable manner, but this requires a commitment to provide those tools and that advice.

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4. PLANT RESISTANCE AND POPULATION GENETICS

4.1 Introduction

The importance of the potato crop in Europe owes much to the improvements that have been made by plant breeders, exemplified by the increase in average yields from about 10 t ha^{-1} in 1850 to the present day average of about 45 t ha^{-1}, an increase due to the combination of improved production techniques and the better potato cultivars produced by breeders [1]. The potato has more characters of economic importance that must be considered by the breeder than any other temperate crop. In Europe, these include resistance to at least twelve major diseases and pests, with data on as many as 60 traits in all being used as selection criteria [2]. Resistance to PCN has enjoyed a position near the top of the list of selection criteria for the last 50 years but perhaps deserves even more emphasis than it has been given recently.

4.2 The use of resistance against PCN

The serious threat to potato production represented by PCN has long been recognised, with *G. pallida* now posing the major threat in the UK. Resistance to *G. pallida* has not yet been deployed effectively in potato cultivars that are widely grown in the UK. However, there are many known sources of resistance to PCN in primitive and wild relatives of cultivated potatoes and some of them have been successfully introgressed into cultivars and breeding lines. Notably, the *Gro-1* (*H1*) gene from *Solanum tuberosum* ssp. *andigena* has provided complete, durable resistance to all known UK populations of *G. rostochiensis* with no sign of loss of effectiveness since the first cultivars to carry it were released in the early 1960s. As noted elsewhere (Sections 3 & 7), the widespread use of such cultivars is one of the main reasons for the current prevalence of *G. pallida*. Breeders have been less successful in producing cultivars resistant to *G. pallida*, having used resistance genes mainly from the wild diploid species *Solanum vernei* and the tetraploid *S. tuberosum* ssp. *andigena*, along with a gene referred to as *H2* from the wild diploid *Solanum multidissectum*. While *H2* is a single dominant gene, other sources of resistance to *G. pallida* are inherited in a quantitative manner. The complex manner of inheritance of the resistance makes it difficult to use in a breeding programme, although that from *S. t. andigena* is somewhat easier to use as the source material is tetraploid rather than diploid as in *S. vernei*. Despite considerable effort, no cultivars are more than about 90% resistant against *G. pallida* relative to non-resistant control cultivars, and the degree of resistance varies with the population of the nematode. Importantly, the resistant cultivars available do not have wide market appeal.

Despite the incomplete nature of the resistance to *G. pallida* in cultivars so far released, they have proved successful in limiting the multiplication of field populations of this species under experimental conditions. When crops were also treated with granular nematicide, *G. pallida* populations were actually decreased, even when the nematicide was used at only half the recommended rate [3]. Thus, their widespread use could be a route to reducing overall nematicide use. However, it has also been shown that *G. pallida* populations become more virulent towards partially resistant cultivars each time that they are grown [4]. To avoid such selection for virulence it is important that different partially resistant cultivars are alternated but, since few growers use even one such cultivar due to poor market appeal, the chances

of two or more cultivars being considered suitable is currently remote. Currently there are no sources of resistance to PCN in potato cultivars used for processing within the UK.

4.3 Durability of resistance

As noted above, the *H1* gene for resistance to *G. rostochiensis* has proved remarkably durable, but there is much evidence that partial resistance to *G. pallida* will not be durable. The theory that alternation of partially resistant cultivars would slow the selection of virulent nematode populations in the field was tested by Beniers *et al.* (1995) [5], who found that this was not true when cultivars contained closely related resistance genes. However, when cultivars containing resistance genes from widely separated sources were alternated, the rate of selection of virulence was retarded considerably. When *G. pallida* populations were deliberately exposed to resistance genes from widely different sources, and attempts made to select for multiple virulence by sequential exposure of the nematode to those resistance genes, the first-selected virulence was sometimes retained and sometimes lost [6].

Thus, although polygenic resistance against *G. pallida* could have a significant impact in PCN management, it is clear that breeders must not rely on only a few sources of resistance but base their resistant cultivars on resistance genes obtained from widely separated sources. Virulent populations will undoubtedly emerge under the effects of selection, but it is difficult to predict how quickly this will happen. Certainly, there will be a dilution effect when the virulent individuals produced by selection cross with non-virulent individuals in the residual population that failed to hatch when the partially resistant cultivar was grown.

4.4 Virulence in *Globodera pallida*

There have undoubtedly been several introductions of *G. pallida* into the UK [7] but it is also certain that there are virulence genes in South American populations of *G. pallida* that have yet to reach the UK. This was implied by results from Phillips and Trudgill (1998) [8], who found that virulence towards a range of partially resistant potato clones varied equally continuously in European and South American *G. pallida* populations, but that South American populations were relatively more virulent than European populations towards certain of the resistant clones.

Folkertsma *et al.* (2001) [9] analysed 226 Dutch populations of *G. pallida* by two-dimensional gel electrophoresis of total proteins and found their genetic diversity to be small. This implies a narrow genetic base for *G. pallida* (i.e. few introductions) in The Netherlands, and the possibility that broad and durable resistance can be found. Interestingly, individual local populations from an area in which close crop rotations are normal showed greater genetic diversity than those from two other areas in which wider crop rotations are more normal. The more intensive production of potatoes has permitted the passage of more generations of *G. pallida* since the original introduction, and the opportunity for greater genetic diversification.

4.5 Population genetics and molecular markers for virulence

The population genetics of PCN within a field, which may contain a mixture of species and virulence types, will have a significant affect on the durability of resistance and is therefore important for the deployment of resistant cultivars, especially those that are only partially resistant [10]. In most fields, PCN is patchily distributed with little movement of nematodes between the patches, which may have distinct genetic structures. In the absence of selection by the growth of resistant cultivars, virulence within a population will depend upon the genetic structure of the original population(s) introduced to the field, random genetic drift and gene flow. Although progress has been made on determination of the genetic variations amongst PCN populations within Europe and the likely number of introductions (see above), we know little of the population genetics of PCN within fields.

To understand such metapopulation dynamics, virulence within a population must be clearly diagnosed and measured. Pathotype schemes based on the identification of populations that multiply on potato lines containing defined resistance genes are fraught with difficulties. The definition of resistance differs between countries, field populations of PCN may contain mixtures of species and pathotypes, resistance to *G. pallida* is polygenic and resistant lines may contain a continuum of resistance genes [11, 12]. Hence, pathotype schemes are not useful and it is more appropriate to refer to virulence types within *G. pallida* [13]. To further knowledge on the genetics of virulence within populations, molecular markers for virulence in nematode populations are required for use in rapid diagnostic tests. Blok *et al.* (2000) [13] summarised the early attempts to develop such markers, pointing out the problems of working with field populations and the use of markers insufficiently tightly linked to the character of interest. More recent approaches to developing such markers have studied the expression of nematode pathogenicity factors involved in the susceptible response and virulence. However, to date such factors have not been identified and the design of reliable markers is not yet possible.

4.6 New sources of resistance to PCN

As more is learnt of the reaction of PCN populations to resistance genes, so the search for new sources of resistance becomes more structured and meaningful. Early searches for resistance were made at a time when little was understood of the range of virulence found in PCN but more recent searches have revealed a large number of sources of resistance, some of which have more potential than others. Thus, Rousselle-Bourgeois and Mugniery (1995) [14] screened over 1600 clones from 52 accessions of tuber-bearing species of *Solanum* and found 135 clones from 23 accessions with resistance to *G. rostochiensis*, and 105 clones from 32 accessions with resistance to *G. pallida*; about 25 clones had resistance to both species. de Galaretta *et al.* (1998) [15] screened 98 accessions from 90 wild *Solanum* species, and a few showed resistance to *G. pallida*, including several previously unreported sources of resistance. Two types of resistance were found in *Solanum sparsipilum* by Mugniery *et al.* (2001) [16], one acting against juveniles soon after they invade roots and the other causing only male adults to be formed. This resistance was effective against very virulent European populations of *G. pallida* but not against a population from Peru. Segregation studies suggested a major gene was involved, and that this gene was different from *Gpa4* already reported from *S. sparsipilum*.

Thus, resistance is known from many sources but its full potential in terms of cultivars that bear high levels of resistance and have good market appeal has yet to be realised in the UK. A more pro-active approach has been taken in the USA, from where only *G. rostochiensis* (two virulence types) are known. Brodie *et al.* (2000) [17] report the release of seed of potato progenies that have resistance to these two virulence types of *G. rostochiensis*, but also carry resistance to the two virulence types of *G. pallida* known to be prevalent in many other potato production areas of the world. This represents a precaution against possible future introductions.

With the report of novel, previously unknown sources of resistance in twelve species from the Hawkes collection of potato germplasm, recently incorporated into the Commonwealth Potato Collection [18], the time is perhaps ripe to exploit all this material. The better, marker-assisted screening procedures that are now available should make the development of useful cultivars significantly easier.

4.7 Quantitative trait loci and molecular markers for resistance

Although quantitatively inherited resistance effective against all pathotypes of both species of PCN has been found, it has proved difficult to deploy effectively in breeding strategies for potato cultivars. Studies with various species of *Solanum* have permitted the mapping of a number of quantitative trait loci (QTLs) involved in PCN resistance. Kreike *et al.* (1996) [19] mapped QTLs for resistance to *G. rostochiensis*, originating from *Solanum spegazzinii*, on chromosomes 3 and 10, whilst van der Voort *et al.* (1998) [20] suggested that a compound locus (*Grp1*, on chromosome 5 in a resistance gene hotspot), containing multiple genes, could explain the quantitative inheritance of resistance to both PCN species. van der Voort *et al.* (2000) [21] suggested that the broad spectrum of resistance to *G. pallida* can be fully ascribed to two loci: *Gpa5* on chromosome 5 explains more than 61% of the resistance and *Gpa6* has a minor effect, explaining 24% of the resistance, with both loci in regions that harbour resistance gene clusters. Bradshaw *et al.* (1998) [22] looked for markers for QTLs for PCN resistance, to help in marker-assisted selection in breeding programmes. The tendency for genes for resistance to nematodes to be clustered with genes for resistance to viruses and fungi was confirmed by Gebhart and Valkonen (2001) [23], who also suggested that genes responsible for polygenic resistance in potato have structural similarity with cloned R genes, and that it should be possible to identify markers very useful for marker-assisted selection in potato breeding.

The basic work on QTL mapping for PCN resistance was further advanced when Bryan *et al.* (2002) [24] used bulk segregant analysis to detect AFLP markers linked to a QTL for resistance to *G. pallida* on linkage group V. This marker has been converted to a single-locus PCR-based marker, which can be used to detect the presence of the QTL in diploid and tetraploid potato germplasm.

Marker-assisted selection is the method of choice for detection of major resistance genes. Although PCR-based markers for the *Gro-1* gene have been available for some time [25], appropriate markers for the quantitative resistance to *G. pallida* with which breeders have to work are still being developed.

4.8 Recommendations

The recommendations given in this section should be considered along with those concerning genetic engineering and the development of novel resistances. The use of molecular methods is important to enhance breeding programmes to develop cultivars with resistance to *G. pallida.*

Benefits in 5 years

- *Maintain germplasm collections to underpin breeding programmes.*
- *Stimulate links between molecular biologists and breeders to ensure the development and uptake of appropriate technologies to improve breeding methods.*
- *Identify molecular markers for virulence and develop rapid quantitative diagnostic tests.*
- *Develop understanding of the population genetics of PCN populations within fields and its impact on the deployment of resistant cultivars.*
- *Increase efforts to breed commercially acceptable* G. pallida *resistant cultivars by conventional breeding techniques.*

Benefits in 5-10 years

- *Use molecular techniques to identify, characterise and possibly modify new sources of natural resistance genes against* G. pallida.

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5. ENGINEERING RESISTANCE TO POTATO CYST NEMATODES

5.1 Introduction

There was some concern at the Forum meeting from representatives of the potato industry that research programmes were too concentrated on the molecular interactions between PCN and their plant hosts and between PCN and their natural enemies. This concern was based on the current unacceptability of GM crops in Europe. However, an understanding of these interactions at the molecular level may not only enable the identification of key genes that could provide novel resistances in modified potatoes but could identify new bioactive compounds for exploitation as nematicides or semiochemicals. Also public opinion may change, especially if GM crops are seen as an alternative to the use of the current nematicides essential for the management of PCN in the UK. This section concentrates on the approaches being considered for the production of GM potatoes with resistance to PCN.

5.2 The compatible response between PCN and their hosts

The invasion of host roots and the establishment of a feeding cell are essential for the successful development of potato cyst nematodes. Eggs of the nematode hatch in response to leachates from host roots and the infective second-stage juveniles may use these compounds to locate roots, which they invade in the zone of elongation behind the root tip. Although, much work has been done to identify hatching stimulants in potato root diffusates (see Section 10), little is known about host location in PCN. However, interference with the function of the amphids through the use of nematicides such as aldicarb or antibodies that bind to amphidial secretions, could affect chemoreception and significantly reduce the invasion of second-stage juveniles. The delivery of peptides or secondary metabolites that affect chemoreception through secretions from the root or rhizosphere bacteria is a largely unexplored area of research.

The nematodes migrate through the root cortex and establish a syncytium alongside the stele. During migration towards the stele, the second-stage juvenile uses its stylet to cut a path through the root cells but it does not feed. The syncytium is formed from the degradation of adjacent cell walls and it is effectively a transfer cell, in which there are cell wall ingrowths that increase the surface area of the cell in support of the rapid transfer of nutrients from the root conducting tissue into the developing nematodes. It is multinucleate and has dense cytoplasm containing numerous mitochondria, plastids, ribosomes, Golgi bodies and extensive smooth endoplasmic reticulum, typical of a highly metabolically active cell. The failure to establish and maintain a fully developed feeding cell results in the death of the nematode and a reduced cell may result in a decrease in fecundity of female nematodes or the development of males that need fewer resources. The size of the syncytium is directly correlated to the size of the female nematode and her egg production. So-called 'feeding tubes' are formed around the stylet and appear to be essential for nutrient uptake. They may have a role in the transport and synthesis of nutrients and may act as a sieve, allowing the passage of proteins 11 kDa but not 22 kDa in size; this would be an important consideration in the delivery of gene products that have to be ingested to be active against the developing nematode (see below). Defence mechanisms that limit syncytial development limit nematode multiplication. The resistant reaction in

plants results in the failure to establish a feeding cell capable of supporting the development of a female nematode.

Thus, there is an intimate relationship between potato cyst nematodes and their hosts, which provides several opportunities to interfere with the molecular events that underpin this interaction. Nematologists around the world use molecular biology as a tool to examine nematode-plant relationships in order to:

Clone and transfer natural resistance genes
Enhance plant defence mechanisms in roots elicited by migrating second-stage juveniles
Identify key molecules in nematode secretions that induce the production of, and maintain, syncytia
Interfere with feeding cell function to affect nematode development and fecundity

The approaches used to create genetically modified plants expressing genes that interfere with nematode development to search for tissue-specific promoters are described below.

5.3 Natural resistance genes

Resistance to PCN conforms to the gene-for-gene hypothesis in which resistance genes (R-genes) in the host are matched with avirulent (*avr*) genes in the parasite. In fungi and bacteria, mutation of the *avr* gene causes the organism to become virulent. The same change in nematodes would result in failure to elicit the nematode recognition and resistance reactions caused by the R-gene. To date, no plant parasitic nematode *avr* genes have been characterised. However, resistance genes, including two active against PCN, have been characterised and cloned and their structure has proved conserved; they contain regions similar to those R-genes for microbial pathogens. Such research will lead to a better understanding of interactions between nematodes and their hosts and manipulation of R-genes could extend their range of activity and provide resistance that could be readily transferred between cultivars. Although the use of the *Gro1* (formerly designated *H1*) R-gene has provided robust resistance to the single virulence type of *G. rostochiensis* in the UK (see Section 3), the widespread deployment of most R-genes has resulted in the selection of virulent nematode populations and durable resistance to *G. pallida* may be dependent on a GM approach.

To date, four R-genes to nematodes have been characterised and cloned (see [1]), including the *Gpa2* gene from potato that confers resistance to a few populations of *G. pallida* [2] and the *Hero* gene from tomato that confers resistance to *G. rostochiensis* and partial resistance to *G. pallida.* It remains to be seen whether transfer of the *Hero* gene into potato provides similar broad resistance. However, the *Mi* gene from tomato did not provide resistance to root knot nematodes when transferred to another solanaceous host, tobacco. Hence, it may prove difficult to use such resistance genes as transgenes for introduction into different crop cultivars unless the factors for the expression of the resistance proteins are understood and the regulatory machinery is present in the recipient cultivar. Surprisingly, the introduction of *Bt* gene for the control of coleopteran pests into a potato cultivar resistant to *G. rostochiensis* resulted in lines that were susceptible to the nematode

[3]. The interaction of genes with such different activities, leading to the loss of expression of the *Gro1* gene, is of interest but it did not occur in all lines that were transformed.

Typical R-genes have a nucleotide binding site and a leucine-rich-repeat (LRR) domain, which are essential for the expression of resistance. Specificity to pathogens, and possibly nematodes, is conferred by the LRR domain and/or the N-terminus of the gene. Engineering these regions may affect recognition of the parasite or pathogen and the expression of resistance to a broader range of virulence types. As R-genes have similar structures they may be more easily recognised using molecular diagnostic techniques than relying on the response of the female nematode, which is time-consuming to measure.

5.4 Plant defence mechanisms

As it migrates within the root, the second-stage juvenile may be recognised and elicit a specific resistance response defined by the R-genes or induce more general defence mechanisms. Cellulases and a pectate lyase are secreted by *G. rostochiensis* juveniles during their migration, the latter enzyme being the first record in an animal [4, 5]. It is not known if inhibitors of such enzymes (see below) would limit root invasion by the nematode. The surface coat of infective animal parasitic nematodes is important in suppressing host responses but its role in plant parasitic species is not clear. However, material is sloughed off the nematode as it migrates through roots and remains on the cell walls in the migration pathway [6] and may protect the second-stage juvenile from the elicitation of plant defences. Many compounds, including secondary metabolites, reactive oxygen, and β-1-4 endoglucanases are produced in response to nematode attack, some within a few hours. Although some are known to affect nematodes and may be involved in hypersensitive reactions resulting in cell death, the control of these processes is complex and has not so far been manipulated to enhance their ability to prevent establishment of the parasites within roots. This would be a long-term research goal.

Pathogenesis related proteins such as chitinases are produced in roots in response to wounding, including that caused by PCN. A range of polypeptides associated with systemic acquired resistance (SAR) has been found in the leaves of potato plants within two weeks of infection by PCN [7]. However, there is no evidence yet that these compounds produced in response to nematode attack affect nematodes in roots.

5.5 The production and maintenance of feeding cells

It is generally accepted that secretions from the dorsal oesophageal gland that enter the plant cell via the stylet are responsible for the induction of the syncytium, and certainly the maintenance of its high level of activity is dependent on the stimulation caused by the continued feeding and stylet probing of the developing nematode. However, other secretions from the surface of the nematode and the amphids are in close proximity to the syncytium and may have a role in its development. Cyst nematodes affect the development of only a few plant cells around their head region and so it is technically difficult to separate nematode-modified cells from uninfected root tissue to study the influence of nematode infection on gene expression. However, methods have been developed and comparisons made between healthy and

infected roots in both susceptible and resistant plants. It is clear that hundreds if not thousands of plant genes are either up- or down-regulated in response to cyst nematode attack and during their development. Many plant genes involved in parasitism have been identified using microarray technologies in which thousands of cDNAs from nematode-infected or healthy roots have been cross-hybridised with labelled cDNAs derived from mRNAs collected from plants at different stages of nematode development. Cross-hybridisation identifies genes that are expressed in nematode-infected tissue and comparison of these genes with those expressed in healthy tissue, resistant roots or the syncytium may lead to the identification of key plant genes involved in parasitism.

A more directed approach has involved the removal of the contents of individual gland cells from different developmental stages of the soybean cyst nematode. Molecular methods were used to construct a library of expressed genes and those with a secretory motif were considered as potential parasitism genes [8]. Such an elegant approach has identified tens of genes that may have a role in the induction and maintenance of the syncytium but the presence of a secretory motif in the gene does not necessarily imply that expression of the gene involves release of a protein into the plant.

Although much will be learnt about the nature of cyst nematode parasitism and the metabolic pathways involved in both host and parasite, these approaches all present a significant challenge for the development of novel resistances to specific nematode pests. At present, plants may be modified to express no more than three transgenes, hence the selection from the many genes known to be expressed in response to nematode parasitism will be critical if interference with their function is to affect nematode development. To be successful, selection will depend on effective gene knockout methods, high throughput bioassays and readily identifiable phenotypes to identify genes that have a significant effect on nematode development. Most work in this area has been done on soybean cyst nematodes and root-knot nematodes and much basic data is being collected on the nature of cyst nematode parasitism, which will have relevance to similar research with PCN that is now being done in the UK.

Early work on novel resistances for cyst and root knot nematodes examined the use of gene products that influenced syncytial function by identifying toxic genes such as barnase, which prevents the functioning of RNA in cells [9]. Even though this gene was coupled to a feeding cell specific promoter, no promoter has given sufficient discrimination from other metabolically active cells, such as those in meristems, to prevent more general phytotoxic effects. Research has, therefore, tended to concentrate on the use of genes that are nematode specific and do not affect plant growth.

5.6 Tissue-specific promoters

Ideally, for PCN management through the use of genetic modification of potato plants, the target gene should be induced by the nematode and deliver active amounts of gene products but have limited expression, possibly only in the syncytium. This requires the identification of specific gene promoters that may be tissue- and temporally specific. Clearly, the use of GM potatoes may be considered less of a risk if the transgene is not expressed in the tuber. Wound-specific promoters have been

identified that are induced by PCN invasion and may be used to express genes that interact with nematode secretions and inhibit migration [10], and a range of other promoters that are induced by nematode attack have been identified through a process known as promoter tagging, some being significantly up-regulated in syncytia [11].

Constitutive promoters do not rely on the presence of the nematode to induce genes and can mount plant defences before the nematode invades roots. The CaMV35S promoter from the cauliflower mosaic virus is the most widely used but this may be down-regulated in the syncytia of *G. pallida* and its activity is reduced in older tissue. Several root-specific promoters have been identified, including the promoter *TobRB7*, which has been engineered to increase its specificity to nematode feeding cells. In general, promoters are available that should minimise the expression of anti-nematode transgenes in potato tubers.

5.7 Transgenes that affect nematode development

The feeding cell could also be used as a delivery system for gene products that interfere with nematode development if fed upon by the parasite. One approach involves the expression of antibodies in plants (plantibodies), first described by Hiatt *et al*. (1989) [12]. Small chain antibodies that bind to PCN secretions, including the feeding tube, could affect the feeding of developing juveniles and adult females [13], but it remains to be seen if sufficient material can be delivered via the syncytium and feeding tube to be nematicidal or affect nematode development. Also, there are likely to be regulatory and public acceptability problems in the expression of modified animal genes in crops. Most success has been achieved through the more general expression of proteinase inhibitors in roots that affect nematode digestion.

An extensive programme at the University of Leeds [see 14] has used proteinase inhibitors to affect nematode feeding and significantly reduce female development and their fecundity in a range of nematode species, including PCN. In detailed studies of the digestion of sedentary nematodes, cysteine proteinases were identified as key enzymes produced in the intestine of feeding females of PCN. As in soybean cyst nematode, these are probably active throughout parasitic development. Serine proteinases have also been recorded as important in the digestion of cyst nematodes. Several proteinases in each class occur in cyst nematodes and each may have a specialist role or be active at different stages in the life cycle of the nematode.

Proteinase inhibitors are part of the plant's normal defences induced by wounding and herbivory and are abundant in seeds and tubers. Hence, they have been widely consumed by humans in their normal diets so are considered to present little risk if used as transgenes. A cystatin derived from rice that is an effective inhibitor of cysteine proteinases has been modified (aspartic residue removed) to significantly enhance its activity. In a field test, the best lines of potato cvs Désirée and Sante transformed to express the cystatin provided > 70% and 85% resistance respectively, relative to the untransformed cultivars [15]. However, when transformed Sante was exposed to a virulent *G. pallida* population, the level of control declined to 51%. In more recent tests, transformed lines of Sante had improved resistance to *G. pallida* and the best line prevented populations of the nematode increasing [16]. In a similar approach, a cystatin gene from the tubers of potato cv. Jersey Royal has provided

significant control (60%) of *G. pallida* populations when expressed in the roots of the same cultivar (Burrows, pers. comm.).

In insects, the use of cystatin has provided only modest control of the target pests because they switched enzyme systems, thus underlining the benefits of stacking different proteinase inhibitors. The co-expression of cysteine and serine proteinase inhibitors in *Arabidopsis thaliana* has provided greater resistance to both root knot and cyst nematodes compared to the expression of either inhibitor alone [17]. These exciting developments indicate that a new source of resistance in potato is available for widespread testing against a range of PCN populations. Proteinase inhibitors appear to be robust and have wide utility amongst several nematode genera of major economic importance compared to the more specific natural R-genes.

A transgenic approach has also been used to improve delivery of the nematicide, oxamyl. Foliar doses of a hydroxymethyloxamyl glucuronide, which is phloem-mobile, were applied to transgenic tobacco expressing β-glucuronidase coupled to a suitable root-specific promoter. The transgene activated the nematicide complex molecule and delivered active oxamyl to the root tips to reduce nematode invasion [18]. The pro-nematicide had low toxicity compared to the parent compound. Other potential sources of genes for novel resistances include the use of peptide mimics of aldicarb, which reduce nematode invasion, or enzymes from nematophagous fungi that degrade eggs shells, and collagenases that affect nematode development. If active, such genes could increase the range of resistance genes to back up the proteinase inhibitors should these not provide durable resistance. All these approaches will require a change in regulations within Europe before they can be widely evaluated in the field.

5.8 Nematode genomics and gene discovery

The availability of the complete genome of the free-living *Caenorhabditis elegans* is an invaluable scientific resource, especially for nematologists. Currently, a research consortium is involved in using RNAi techniques to specifically knock out each of the 20,000 genes that make up the genome and to examine the impact on the nematode. Studies on the genome of the soybean cyst nematode suggest that it is organised in a very similar way to that of *C. elegans* and, presumably, that of PCN will also be similar. Such synteny will help in the identification of genes of similar function by targeted searching in the genome. Comparative studies with plant parasitic species will aid the identification of putative parasitism genes and of key genes required for nematode development. Interfering with the expression of such genes through a transgenic or chemical approach could provide novel mechanisms for nematode management.

An increased knowledge of the genome of *G. pallida* is essential for the identification of genes that affect hatch, diapause, host location and reproduction, and would greatly increase options for management, as well as our fundamental understanding of a nematode parasite. Although large scale sequencing capacity and bio-informatics capabilities are available in the UK, it would require a major collaborative project to sequence the entire genome. However, more modest collaborations amongst the UK nematology community could provide significant new information. Expressed sequence tags (ESTs), which may be collected at different stages in the development

of the nematode, are generated from mRNA and represent expressed genes. Bio-informatic procedures can be used to compare EST datasets and, through comparison with datasets from other nematode species or other organisms, genes involved in key nematode processes can be identified. An international, collaborative project, the "Nematode Net", aims to generate >300,000 ESTs from about 20 nematode species (mostly animal parasites), which will be a major research resource for the identification of nematode genes. Currently, *c*. 54,000 ESTs from nematodes in the family Heteroderidae are in the public domain but <8000 are from PCN.

The construction of genetic maps for PCN is problematic because of the difficulties in producing defined (inbred) populations of the nematodes for crossing experiments and the length of the life cycle. However, methods of physical mapping are important tools for the localisation of genes within the genome and the identification of regions involved in selection processes. The process of HAPPY mapping uses PCR techniques based on primers derived from ESTs. Such a map would provide a backbone on which to place specific genes in order to locate them within the genome and would be a great asset to gene discovery.

5.9 Health and safety issues relating to GM potatoes

Transgenic crops have had the most rapid uptake of any technology in agriculture worldwide but within Europe still cause much concern over their perceived health, environmental, socio-economic and ethical impacts. Recent research findings from the extensive evaluations of herbicide-tolerant crops have clearly demonstrated that these new crops, which require changes in their agronomy, may have different environmental effects to their conventional counterparts and that each new crop must be assessed individually. The only GM potatoes grown that constitutively express nematode resistance have been those containing proteinase inhibitor transgenes and some preliminary research has been done to study their environmental impact. Changes in the numbers of soil microarthropods and free-living nematodes were not significant when potatoes expressing a cysteine proteinase inhibitor were grown, and changes in the structure of the soil microbial community were no greater than those resulting from nematicide use [19]. When aphids were fed on similar plants, the numbers were not different from the numbers found on control plants, whereas treatment of the plants with nematicide reduced the number of aphids dramatically [19].

The cystatins used in the GM potatoes from the Leeds programme are naturally found in rice seed and presumably have been consumed for generations; they are destroyed by gastric juices and cooking. Oral toxicity experiments with the modified cystatin have demonstrated no toxic effects [20]. As introduced pests, PCN are confined to cultivated fields and so resistance to them provides no selective advantage in wild plant communities but, if the gene transferred to wild Solanaceae, its broad activity might affect other parasitic nematodes. However, the clonal propagation, short pollen transfer distance, and the failure to hybridise with wild Solanaceae minimise the risks of gene escape in the UK. Most observations to date have involved dietary experiments incorporating the inhibitor protein or have studied impacts on non-target organisms in field tests of proteinase inhibitor transgenes using a constitutive promoter; the use of a root-specific promoter might further reduce such impacts and also reduce the expression of the gene in tubers. Consumer antagonism towards

transgenic technology may not be soundly based but, in the case of PCN management, it should be considered as a measure that would greatly decrease dependence on nematicides, including the partial soil sterilants.

5.10 Recommendations

At the time of submission of this report there is much public concern, in Europe, over the commercial use of GM crops and of the acceptability of foods derived from them. This must not slow the scientific progress being made in the use of molecular techniques to understand the parasitism of PCN and the identification of new methods of management that are likely to be more environmentally benign than current practices. A shared recommendation from this section is in the identification and possible modification of natural R-genes active against *G. pallida*, which was also identified in the Section on Resistance. Other specific recommendations are given below.

Benefits in 5 years

- *The GM potato lines expressing proteinase inhibitors should be tested more widely in containment and field conditions to evaluate their durability against different PCN populations and the transgenes transferred to commercially acceptable cultivars.*

- *Finance (from more than one agency) should support a UK Consortium of molecular nematologists to construct a physical map of the genome of G. pallida, in collaboration with a centre with sufficient genomics and bioinformatics support. The map should be placed in the public domain to maximise its exploitation for gene discovery.*

- *Research on the factors influencing public attitudes to GM technology is urgently required and should lead to improved acceptability of transgenic potatoes that may reduce nematicide usage.*

Benefits in 5-10 years

- *Identify, characterise and inhibit the activity of genes involved in important aspects of the life cycle of PCN, which may be exploited through transgenes, new nematicides or semiochemicals. This will provide additional novel resistance genes should the proteinase inhibitors not prove durable and new bioactive compounds should GM crops remain unacceptable in Europe*

5.11 References

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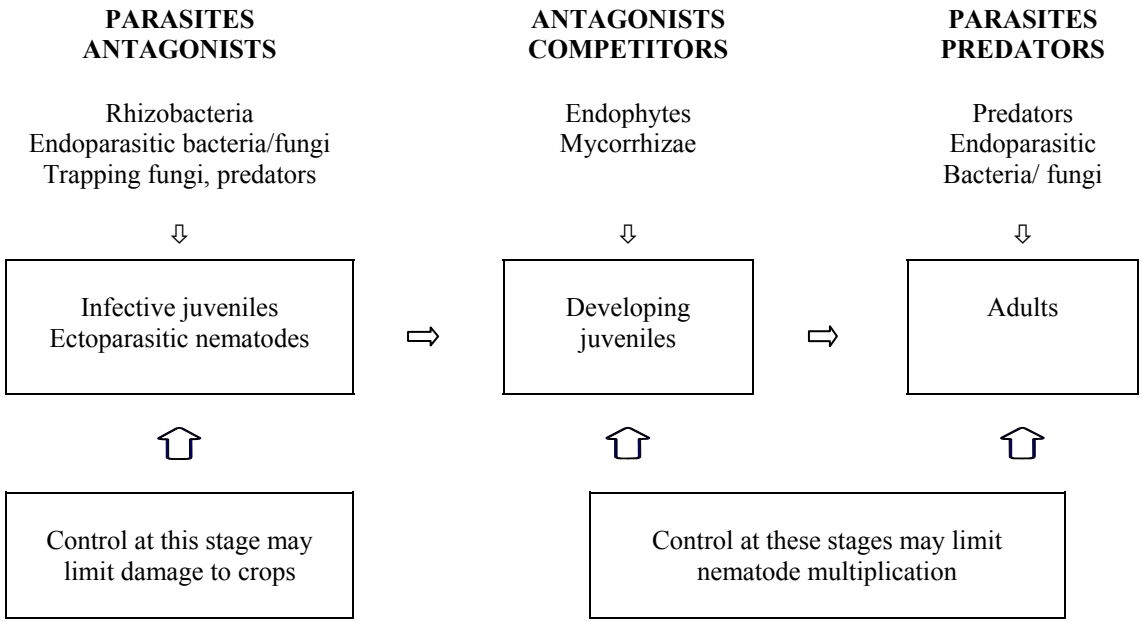
6. BIOLOGICAL CONTROL

6.1 Introduction

Although, many predators of nematodes have been identified amongst protozoans, nematodes, enchytraeids, insects, and mites, none is specific and none has been demonstrated to have a significant effect on the populations of a specific nematode pest. Hence, biological control of potato cyst nematodes is concerned with a range of specialist and generalist microbial competitors, antagonists and parasites (Figure 6.1). The microbes that attack active second-stage juveniles must have specialised infection structures such as traps or adhesive spores or produce toxins to immobilise their prey. In contrast, a range of relatively unspecialised antagonists, pathogens and parasites are known to attack the sedentary females and eggs in the rhizosphere [1, 2].

Figure 6.1 Interactions between different microbial natural enemies of Potato Cyst Nematodes and their likely effects on control.



6.2 Microbial pathogens and antagonists of nematodes

Several organisms may be involved in soils that are suppressive to PCN (see below) but this remains to be proven. Although, PCN are introduced pests to Europe, soils in the UK contain isolates of all the major agents considered for the biological control of these pests. Biological control agents may be practically exploited in three ways:

As biopesticide products based on specific organisms for application to soil
As a source of bioactive compounds for use as new nematicides or for expression as novel resistance sources in transgenic plants
As part of the soil microbial biodiversity that should be managed to create soils suppressive to PCN

6.3 Organisms that attack the mobile stages of PCN

PCN affect crop yields directly through the alteration of the morphology of the root system that results from their feeding on, or invasion of, root tissues. The migratory, second-stage juveniles cause this damage and, hence, for natural enemies to reduce most yield losses they must have appropriate mechanisms for killing, or affecting the behaviour of, active nematodes to reduce their ability to invade roots. As the control of the infective stages is unlikely to result in post-cropping population reduction, effective natural enemies that are active at this stage must at least be capable of reducing nematode pre-cropping populations to densities below the economic damage threshold.

6.3.1 Antagonistic rhizobacteria

Isolates of *Agrobacterium radiobacter, Bacillus subtilis* and *Pseudomonas* spp., familiar as antagonists of soil borne bacterial and fungal diseases of plants, also have potential as biological control agents for nematodes. The modes of action of these bacteria differ, but direct effects on egg hatch and nematode mobility, and indirect effects such as alteration of root exudates and induced resistance, have all been demonstrated. A reduction of root penetration by *G. pallida* in the presence of *Bacillus sphaericus* or *A. radiobacter* is related to the development of systemic resistance induced by the presence of the bacteria in the rhizosphere [3, 4].

The most promising isolates of rhizobacteria have reduced invasion by about 60-70% [5] so are unlikely to reduce nematode multiplication significantly [6]. Their significance in the reduction of nematode damage to plants will depend on their ability to reduce nematode invasion for a sufficient period of time (2-3 weeks) when soil infestations are above the damage threshold. At present, these bacteria have not been evaluated at a range of nematode densities. Also, it seems unlikely that seed treatments can provide sufficient inoculum to protect roots for more than a few weeks and their use on tolerant crop cultivars may be necessary.

6.3.2 Bacterial parasites of the mobile stages of PCN

Although several bacteria may be antagonistic to nematodes, only *Pasteuria* spp. have been studied as potential biological control agents. Three gram-positive bacterial species, *P. penetrans*, *P. nishizawae* and *P. thornei*, have been distinguished in this genus and these species parasitise a range of nematodes; *P. nishizawae* is from soybean cyst nematodes and spores of this species and of some populations of *P. penetrans* attach to PCN. All are obligate parasites that produce spores that attach to the cuticle of nematodes. Most research has been done on *P. penetrans* on *Meloidogyne* species. It may be a causal agent in root-knot nematode suppressive soils and has potential as a biological control agent [7]. The spores of *P. penetrans* attach to the second-stage juvenile and germinate within a few days of it beginning to feed within the root. *Pasteuria penetrans* is a true parasite in that the growth and development of the nematode host appears to be unaffected until the onset of egg production. Infected nematode females complete their development but produce no or few eggs and may contain up to two million spores which are released into the soil as the nematode cadaver decays. The spores are resistant to desiccation and high temperatures and may survive several years in soil [see 7]. Second-stage juveniles of root-knot nematodes heavily encumbered with spores may fail to invade roots [8, 9].

Pasteuria penetrans has much potential as a biological control agent and a commercial product (Nematech Ltd., Tokyo) has been produced for control of root-knot nematodes. The spores of *P. penetrans* are extremely robust and have a long shelf life (several years). However, mass production remains difficult and individual isolates of the bacterium have restricted host ranges that may limit commercial development.

6.3.3 Nematode-trapping fungi

Different trapping structures, which may or may not be adhesive, are formed by this group of fungi. In soil treated with organic amendments, activities of nematode-trapping fungi are more related to the state of decomposition of the organic matter than to nematode density [10]; there is a succession of fungal species, each with a limited period of activity [11]. The relationship between the saprophytic phase, the development of traps and the parasitic habit are poorly understood even in the most intensively studied species, *Arthrobotrys oligospora*. The second-stage juveniles and males of PCN are susceptible to these fungi. However, the rather complex interactions between the nematode and organic matter in soil have meant that it has proved difficult to manipulate individual isolates of these fungi to produce traps at the time that the second-stage juveniles are migrating towards the roots and when adult males are active in the rhizosphere. Although some commercial products based on these fungi were produced in the late 1970s, none was successful.

6.3.4 Fungi with adhesive spores

The most studied endoparasites, such as *Drechmeria coniospora*, *Hirsutella rhossiliensis* and *Verticillium balanoides,* produce small spores that attach to the nematode cuticle. Although there is no saprophytic growth in soil, these fungi can be cultured on laboratory media. *Drechmeria coniospora* also infects nematodes via adhesive spores that attach specifically around the amphids, which are the anterior chemoreceptors. As a result, the chemotaxis towards food sources by spore-encumbered nematodes is significantly impaired [12]. These fungi do not compete well with the residual microflora in soil and have proved difficult to exploit.

6.3.5 Pathogens of females and eggs

The saccate females of PCN develop on the root surface and are exposed to a range of non-specialised pathogenic fungi for periods of a few days to several weeks. More than 150 species of fungi have been isolated from the cysts, females or eggs of cyst nematodes but the parasitic status of fewer than 10% has been tested [13]. *Pochonia chlamydosporia, Paecilomyces lilacinus* and *Plectosphaerella cucumerina* are the most abundant fungi attacking the females and eggs of PCN [14]. Observations on sugar beet plants infected with the cyst nematode *H. schachtii* indicate that different fungi are active at different times of the growing season; more first generation females and eggs are infected with *P. chlamydosporia* whereas *Cylindrocarpon destructans* is the dominant pathogen in later generations [15]. In soils that are suppressive to the cereal cyst nematode, *H. avenae* (see below), there is much variation between the different isolates of *P. chlamydosporia* taken from individual nematode eggs from the same soil. The role of such variation in the regulation of

nematode populations is unknown. In general, isolates of these fungi differ significantly in their ability to parasitise the eggs of different nematode species. Some fungi and bacteria colonise plant tissue but do not cause lesions or other symptoms and are referred to as endophytes. Such organisms may be mutualistic if they protect the plant from herbivores or pathogens and parasites. These fungi may reduce the numbers of nematodes developing in roots, but it is not clear if this effect is due to toxin production as such fungi may also compete for space in the roots, alter the physiological state of root tissue or colonise feeding cells to the detriment of nematodes [16]. Also, mycorrhizal fungi have been widely reported to improve the growth of nematode-infected plants and, in some cases, to reduce nematode infestations.

Most of the fungi isolated from nematode eggs have been readily grown on a range of artificial media and two species in particular, *P. chlamydosporia* and *P. lilacinus*, have been much studied and their potential as biological control agents assessed. The latter species is currently being commercialised (Bioact®, Prophyta GmbH, Malchow, Germany), for use against cyst and root-knot nematodes.

6.4 Suppressive soils

Most nematode populations are regulated by the natural enemy community [1] but only in suppressive soils is its impact of practical significance (Table 6.1). The suppression of nematode multiplication on intensively cropped susceptible hosts by biotic factors in soil was first demonstrated by Gair, Mathias and Harvey (1969) [17] with the cereal cyst nematode. Suppressive soils contain microbial communities which have increased in size to densities that prevent nematode populations multiplying by reducing the development of juveniles, fecundity of females and the survival of all stages. Often, the causal agents of nematode suppression have tended to be only one or two species of nematophagous fungi. In intensive cropping systems, particular species of natural enemy appear to be selected from the community in the continued presence of a nematode host. However, in some soils several natural enemies may be involved but it is difficult to demonstrate their significance in the regulation of nematode populations. Suppressive soils are induced as nematodes are usually abundant in the early stages of their development but once established they have provided the most sustainable method of nematode management in intensive agricultural systems.

Table 6.1 Characteristics of nematode-suppressive soils that affect their exploitation in the biological control of plant-parasitic nematodes.

- suppressive soils have provided the most sustainable methods of nematode management in intensive agriculture
- suppressive soils provide a valuable source of potential biological control agents
- soil amendments and some crop cultivars may be used to alter microbial communities in the rhizosphere to the detriment of nematode pests
- control is often specific to one nematode pest species and minimises impacts on non-target organisms
- the natural enemy community is often diverse
- pest control is often slow to establish and may take 3-5 cropping cycles

Populations of the cereal cyst nematode, *Heterodera avenae,* usually decline after the fourth or fifth cereal crop to non-damaging infestations because females and eggs of the nematode are parasitised in the rhizosphere by *Nematophthora gynophila* and *Pochonia chlamydosporia*. The decline phenomenon is widespread in northern Europe and has been extensively studied [18]. Changes in the densities of spores of both fungi in soils were inversely correlated with the abundance of the nematode, and fungal populations required at least 3 years to reach densities that controlled the nematode on susceptible crops. Populations of the nematode, which reached 200 eggs g^{-1} soil in some microplots, decreased to < 5 eggs g^{-1} soil in 4 years despite the continuous cropping of susceptible spring barley.

Similar decline phenomena caused by the activities of nematophagous fungi have been reported in fields infested with other cyst nematodes. Nematode suppressive soils have been developed in small plots infested with a range of cyst species, including PCN [19]. Soils suppressive to PCN probably do not develop in growers' fields because potato crops are grown in rotations and there is much mixing of rhizosphere soil at harvest, which would slow the build-up of natural enemy populations to effective levels. However, fungi may be found in significant proportions of PCN eggs in some soils [20].

6.5 Bioactive compounds

Several rhizosphere bacteria and nematophagous fungi produce metabolites *in vitro* that may inhibit the hatch of eggs and the mobility of the second-stage juveniles. However, these compounds may not be produced at effective concentrations within a cyst or on the surface of roots. Several compounds have been identified that may lead to the development of novel nematicides or proteins that could be expressed in transgenic plants to provide nematode resistance.

An ovicidal factor produced by a strain of *Pseudomonas aureofaciens* has been characterised in terms of its amino acid and nucleic acid sequences [21]. The metabolites produced by the fungus *Myrothecium* spp. have been commercialised as the nematicide DiTera® (Valent Biosciences Corp., Chicago).

Bioactive compounds have been isolated from *in vitro* cultures of *P. chlamydosporia* and *P. suchlasporium*. Such egg parasitic fungi also secrete a range of enzymes that degrade nematode egg shells and cuticle. Immature eggs exposed to a serine protease from *P. lilacinus* failed to develop but the enzyme increased the hatch of more mature eggs. A serine protease from *A. oligospora* immobilises nematodes and hydrolyses proteins in the cuticle of the nematode. Research to express this enzyme in transgenic tobacco to create a novel form of resistance is under way [22].

6.6 Biological control strategies

Biological control of soil-borne diseases has had most success in situations where the target site is readily accessible and can be treated with inundative treatments and when short-term protection results in significant yield benefits [23]. PCN present more intractable control problems and there is usually a need to protect developing root systems for several weeks. Too often the development of biological control

agents has depended on empirical tests [1] but careful selection of active isolates and an understanding of the factors affecting the epidemiology of the agent and pest are essential for the development of successful strategies utilising biological agents.

Few studies have attempted to measure the population densities of the microbial agents in soil and the saprophytic phases of the facultative parasitic fungi are poorly understood. An understanding of the role of bacteria and fungi in the regulation of PCN populations requires detailed knowledge of the population dynamics of both the natural enemy and the host. Such information would underpin the development of biological control strategies but is currently lacking. This situation results from difficulties in the quantification and visualisation of microbial agents in the rhizosphere. The importance of density dependence, transmission rates and threshold values in the interaction between the obligate parasite *Hirsutella rhossiliensis* and the cyst nematode *H. schachtii* have been demonstrated [24]. The epidemics established in microcosms using this model system did not have explosive dynamics and transmission rates are low unless the nematode is present in high densities. There is an important distinction between the suppression of nematode populations that may be caused by abiotic, density independent factors, such as nematicides or soil texture, and the regulation of nematode populations by density dependent agents with feedback mechanisms. Although it is known that potato cultivars differ in their susceptibility to nematode attack, the importance of nematode density and multiplication on the efficacy of biological control agents has rarely been considered. Molecular tools to diagnose and quantify nematophagous fungi in soil are greatly improving our understanding of their epidemiology [25].

6.7 Selection, mass production, formulation and application of selected agents

Nematode suppressive soils are likely to be useful sources for the isolation of potential control agents [26, 27]. Although the pathogenicity of trapping fungi and egg parasites can be evaluated in tests on agar, a screen to assess growth in the rhizosphere is essential. Isolates are selected not only on the basis of their activity against nematodes; other factors, such as ease of production, host range, development of resting structures and growth in the rhizosphere must be considered before testing in soil. A tiered screening process was designed for the selection of isolates of *P. chlamydosporia*, which involved tests for growth in the rhizosphere, chlamydospore production and pathogenicity and enabled almost 90% of the isolates to be discarded before further evaluations were made in the glasshouse [28].

Fewer than ten organisms have been tested for control of nematodes in the field and, as a consequence, limited efforts have been made to optimise methods for their production, formulation and application. However, much can be learnt from the development of other microbial biological control agents [see 29]. Most rhizobacteria and nematophagous fungi are able to grow on artificial media, including in liquid fermentation, but resting structures such as chlamydospores are often not produced in large numbers in submerged culture. These resting structures often enable the agents to be readily handled and provide reasonable shelf lives without the complex formulations required for organisms applied as vegetative cells, hyphae or conidia. The large bulk of soil (2500 t ha^{-1}) that may need to be treated for control of plant-parasitic nematodes and the large densities (10^3-10^6 g^{-1} soil) required for effective

nematode control [28] are likely to make broadcast treatments uneconomic and the restricted placement of inoculum essential.

Rhizobacteria and endophytes have the advantage that they may be applied as seed treatments and will proliferate and spread in the rhizosphere or within the root, protecting the plant from nematode invasion [6]. Granular formulations have been applied to soil in low-pressure drip irrigation systems. However, most nematophagous fungi have limited abilities to colonise non-pasteurised soil and, as with obligate parasites that do not proliferate, successful establishment in the rhizosphere will require thorough mechanical incorporation.

Several nematophagous fungi have been added as active mycelia growing on colonised media that are often waste products, which provide an energy base to help establish the fungi in soil but which may attract competitive saprophytes that reduce proliferation of the parasite. As a consequence, nematode control is variable and any reductions in nematode populations may also be due to the soil amendment effect of the organic matter as well as any direct effect of the fungus [1]. In general, the combined use of a soil amendment and a biological control agent has involved large amounts of organic matter (>1 t ha^{-1}) and appears to be an inefficient method of application that could only be considered for use on small areas near to the site of production.

6.8 Integrated control strategies

Two approaches have been used for the exploitation of nematode natural enemies: the use of methods to increase the activity of the indigenous flora and fauna, or the application of selected organisms as biological control agents. In either approach it has proved necessary to use additional control measures, as biological agents alone rarely provide control levels that can be practically exploited (Table 6.2). In practice, the manipulation of the indigenous antagonists has been largely restricted to the use of soil amendments and crop rotation. Chitin soil amendments have been used to increase chitinolytic activity within the soil microflora, especially actinomycetes, and have significantly reduced populations of root-knot nematodes. However, the amount of material required and its cost will usually restrict the use of such materials unless suitable green manure crops can be incorporated into the cropping cycle [30].

The most promising isolates of rhizobacteria may reduce the invasion of damaging populations of second-stage juveniles. Those agents, such as *P. penetrans*, *P. chlamydosporia*, and *P. lilacinus*, that attack nematode females and eggs should be applied to non-damaging infestations of nematodes as they will not prevent initial damage to susceptible crops.

Table 6.2 Integration of biological control agents (bcas) with other management strategies.

- Methods to reduce nematode populations*:
- Crop rotation with non- or resistant hosts.
- Combined use with nematicides.
- Application of bcas after partial soil sterilisation

- Methods to increase microbial activity:
- Soil amendments† and green manure crops.
- Selected plant cultivars with antagonistic rhizosphere microflora.

*Methods that reduce nematode densities in soil may reduce the efficacy of obligate biological control agents.
†Soil amendments must be carefully evaluated as they may directly reduce nematode infestations in soil and also reduce the effectiveness of some bcas by increasing competition with the general soil microflora.

Other measures successfully used in combination with biological control agents include partial soil sterilisation [31], solarization and nematicides [32]. Kerry (1987) [33] suggested that biological control agents that parasitised nematode females on resistant hosts could slow rates of selection of virulent populations.

The use of biological control agents, even in integrated strategies, still requires the production of reliable data to demonstrate efficacy in the field. Commercial development will only be achieved if successful agents can be produced cost effectively. There is also a need to assess the impacts of biological control agents on non-target organisms and to develop methods to monitor them after their release. Much research and development are still required before even the most studied organisms can be applied on a large scale.

6.9 Recommendations

None of the natural enemies of PCN have provided > 80% control; most reductions in populations have been considerably lower and it seems unlikely that more effective agents await discovery. As a consequence, there is a need to integrate biological control agents with other control measures and to understand the factors affecting their activity in soil to make control more predictable.

Benefits in 5 years:

- *Determine if selected microbial agents can increase decline rates of PCN between potato crops and slow rates of selection of virulent populations on partially resistant cultivars.*

- *Assess whether sustainable control of PCN is best achieved through the manipulation of the residual microflora in potato land or through the application of selected agents.*

Benefits in 5-10 years:

- *Through the development of public-private partnerships, develop selected isolates of fungi or bacteria as biological control agents against PCN and related cyst nematodes.*

- *By studying the infection processes and antagonism of selected agents identify and characterise bioactive compounds for use as novel nematicides or sources of plant resistance.*

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7. DECLINE RATES AND CROP ROTATION

7.1 Introduction

Most management strategies for plant parasitic nematodes depend on crop rotations of non- or resistant host crops grown in sequences of fully susceptible crops. The interval between susceptible crops depends on the rates of multiplication of the nematode on such hosts, the rates of decline of nematode populations under other crops in the rotation and the socio-economic pressures to grow the susceptible crop. These rotations may need to be long but, since the 1960s, crop rotations have been shortened through the use of nematicides and resistant cultivars.

For PCN, the narrow host range amongst solanaceous crops has meant that these pests may be effectively controlled by crop rotations. In the UK, other solanaceous crops, such as tomato and aubergine, are not grown in the field and solanaceous weeds, such as deadly nightshade, are easily controlled and are rare in arable fields. Therefore, all arable crop rotations are effective in reducing nematode infestations in soil as long as volunteer potatoes are effectively controlled (see below). The effectiveness of crop rotations is countered by the longevity of the nematode in soil and the low spontaneous hatch of PCN eggs, which in the absence of a host crop is typically only c. 30% per annum for *G. rostochiensis* and 20% per annum for *G. pallida*. Hence, if soils become heavily infested, it will take many years for populations to decline to non-damaging levels (Table 7.1). Rotations of potato crops of more than nine years may be necessary to guarantee profitable crops [1]. However, if infection of fungal parasites of eggs (described in Section 7) is included in the equation decline rates may be significantly enhanced. Hence, if 50% of eggs were parasitized each year in an initial population of 50 eggs/g soil with a 30% spontaneous hatch, non-damaging populations could be achieved in 3 years and not 7 years as predicted (Table 7.1). In Northern Ireland, PCN is largely managed by crop rotation; land infested with PCN is scheduled and the growth of potatoes on such land is illegal. Some land has remained scheduled for up to 50 years [2] but it is unlikely that in the absence of host plants PCN eggs remain viable for > 25 years.

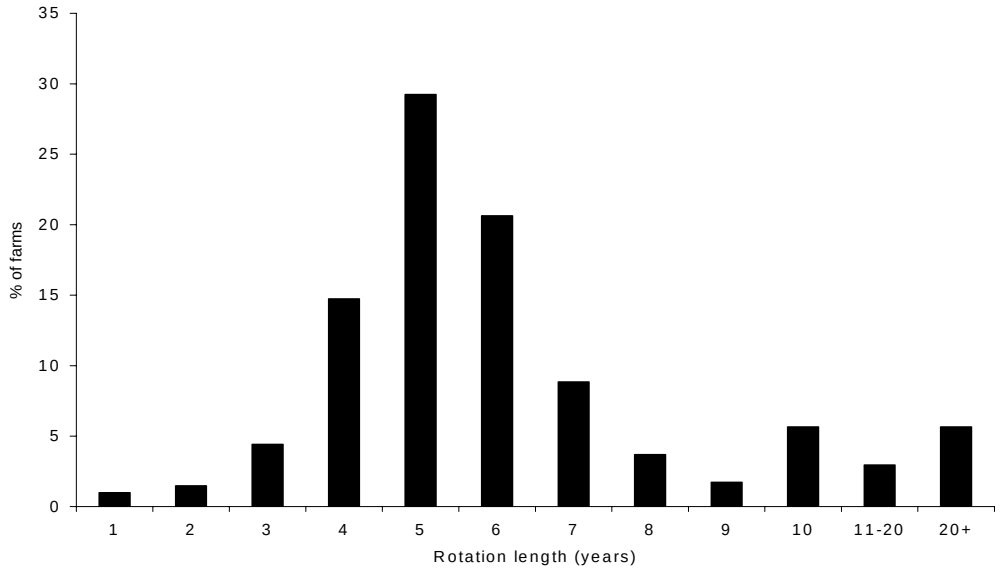
Table 7.1 Estimated rotation length (years) needed for potato cyst nematodes to decline to five viable eggs per gram of soil at different decline rates†.

Percentage decline per annum	Infestation (viable eggs/g soil)				
	50	100	200	400	800
50	3	4	5	6	7
40	5	6	7	9	10
30	7	8	10	12	14
20	10	13	17	20	23
10	23	28*	34*	-*	-*

†At 5 eggs/g soil, little or no damage is likely to a potato crop. Underlined figures indicate the limits of acceptable rotation length for most ware potato growers.
*Eggs that are >25 years old are unlikely to contain viable, infective juveniles. (After Whitehead, 1995 [3]).

Potato production has become a specialist activity that involves considerable capital costs in machinery and storage facilities, so growers want to shorten rotations to maximise returns on their investment. Economic pressures have meant that growers include potatoes in their rotations more frequently than is recommended and more than 50% of growers currently crop potatoes in rotations of < 5 years (Figure 7.1 from Minnis *et al.*, 2002 [4]). Typically, on a mineral soil heavily infested with *G. pallida* (100 eggs/g soil), it may take 13 years for the population to decline, without the use of nematicides or resistant cultivars, to a level that is safe to grow a susceptible potato crop. However, much of the early data on decline rates is difficult to interpret because population estimates were based on cysts and not eggs, and the age and species structure of the populations was often not defined.

Figure 7.1 Current length of crop rotations used by potato growers in England and Wales. (After Minnis *et al.*, 2002 [4]).



7.2 Effect of PCN species and soil conditions on rates of decline

On average, *G. rostochiensis* has a 40% greater spontaneous hatch than *G. pallida* and is more readily controlled by crop rotation [5], but differences between nematode populations and soil conditions greatly affect this hatch. The rates of decline differ significantly in different countries with rates > 50% being recorded for *G. rostochiensis* in New Zealand, Morocco, Ecuador and Bolivia. These rates of decline are generally larger than those observed in Europe, except in the potato growing regions around the Mediterranean where soil temperatures in summer may reach lethal levels. High decline rates of PCN were found to occur in free-draining soils and soils with higher organic matter and clay contents [6] but the reverse may be true in England [7].

Nematodes use their lipid reserves much more rapidly (x 80) once their dormancy has been broken and the infective second-stage juveniles become active in soil. However, even in the dormant stage in eggs, lipid reserves are slowly utilised at a linear rate. In conditions in South America the rate of utilisation may be as high as 23 % per annum [8] but in the UK rates are slower and it may take dormant juveniles of *G. pallida* 7.5

years to reduce their reserves by 50% [9]. The infection of plant roots by second-stage juveniles of PCN was much reduced when lipid reserves were depleted by > 50% of their original amount. The initial reserves in individual infective juveniles depend on host cultivar and on day length, being less in short day conditions. In S. America, the lipid content of second-stage juveniles was significantly different in nematodes that developed on different cultivars [10] and judicious choice of crop cultivar may enable crop rotations to be reduced to 4 years, after which juveniles would retain insufficient lipids to be infective. The scope to manipulate lipid reserves in UK conditions to the detriment of juvenile survival is unknown but differences in the lipid content of second-stage juveniles produced on first or second early or main crop potatoes were insignificant.

In general, the decline in PCN populations between crops is considered largely to be due to spontaneous hatching and not to mortality factors, which were considered to reduce populations by < 15% per annum [11]. Both intermittent exposure to potato root diffusates, at concentrations that are insufficient to cause hatch, and the enzymes secreted by bacteria within the cyst, may increase egg shell permeability and affect the viability of eggs but the importance of such effects has not been quantified [11]. Similarly, the importance of the wide range of fungi isolated from PCN cysts and eggs (see Section 6) in reducing the longevity of PCN in soil is unknown but the fact that decline rates are density independent suggests the indigenous microflora in most potato land does not have a significant effect on the viability of encysted eggs. However, *Paecilomyces lilacinus*, *Plectosphaerella cucumerina* and *Pochonia chlamydosporia* added to soil significantly reduced the numbers of eggs in cysts over a 13 week period (S.D. Atkins pers. comm.).

7.3 Importance of crop cultivars and volunteer potatoes on decline rates

Stone *et al*. (1973) [12] demonstrated that non-host cropping with cereals, grasses and a range of horticultural crops had no effect on the rates of decline of *G. pallida*, which varied between 15 and 24% per annum over a 7 year period. Regular cultivation of infested soil increased decline rates, which might be expected to be slower under grass [2]. Little further work has been done on non-host crops that may influence rates of decline but in a glasshouse test several lines of non-host crops, including lupins and barley, significantly increased the rates of decline [14]. Although such tests tend to over-estimate decline rates compared to what happens in the field, where root densities are much lower, non-host crops that stimulate hatch would be important as trap crops, offering a safer procedure than using host crops (see Section 10). Volunteer plants at densities of > a plant m^{-2} can maintain or increase PCN abundance where infestations are low. Volunteers have little effect on large nematode densities but, when they occur in crops of GM herbicide tolerant sugarbeet, the late removal of weeds (including volunteer potatoes) using glyphosate can prevent PCN population increase [15].

7.4 Opportunities for manipulating decline rates

As decline rates in different PCN populations vary from 10 to 50%, the ability to predict or manipulate these rates could have important management consequences. Therefore, it is essential to understand the factors that affect decline rates between potato crops and identify those that may be managed by the grower. These include

the application of antagonistic fungi and bacteria to soil to increase in-egg mortality rates and a re-evaluation of non-host and antagonistic cultivars on hatching. In the longer term, understanding the mechanisms that affect lipid deposition in the second-stage juveniles may provide new methods to reduce their prolonged survival and their infectivity.

7.5 Recommendations

Benefits in 5 years

- *Assess if selected bacteria and fungi can be increased in the rhizospheres of PCN non-host crops and increase rates of decline.*

- *Evaluate non-host cultivars to assess their ability to increase PCN egg hatch and their suitability for incorporation into potato rotations.*

Benefits in 5–10 years

- *Identify factors that influence the deposition of lipids in second-stage juveniles and attempt to minimise the lipid content of nematodes to reduce their long term survival and infectivity.*

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8. REVIEW OF NEMATICIDES FOR THE CONTROL OF PCN IN THE UK

8.1 Introduction

Many factors affect the use of nematicides for control of PCN in the UK, ranging from political through economic to biological. All have some influence on the direction of research into improving the efficacy of chemical nematicides and research into understanding their behaviour in the soil, and consequent effect on PCN. In summarising the current use and understanding of nematicides for PCN control, information and opinions put forward at the open forum, and from informal discussions with researchers and representatives from industry, are considered along with information from academic and farming press publications.

To maintain the current level and area of potato production in the UK, nematicides are an essential tool for the control of PCN. These chemicals are either soil fumigants that are non-specific in their action, or are granular in formulation with a mode of action more targeted towards nematodes. In general, agronomists, advisors and growers understand the advantages and disadvantages of the nematicides commercially available and there is sufficient competition between the different products to generate continual support and advice from the chemical producers concerned. Currently, there are four granular nematicides and one fumigant for use on potatoes in the UK (Table 8.1).

Table 8.1 Nematicides currently available in the UK for the control of PCN

Active ingredient	Type	Trade name	Company	Hectares treated*
	Granular			
aldicarb	carbamate	Temik	Bayer	8357
ethoprophos	organophosphate	Mocap	Bayer	1357
fosthiazate	organophosphate	Nemathorin	Syngenta	2231
oxamyl	carbamate	Vydate	Du Pont	7453
	Fumigant			
1,3-dichororopene (1,3-D)	halogenated hydrocarbon	Telone II	Dow	730

* in UK per annum - Source: David Richardson, PSD

8.2 The commercial advantages and disadvantages of granular nematicides:

Advantages

- All the granular pesticides used for the control of nematodes in the UK are soil-incorporated acetylcholinesterase inhibitors and, therefore, more targeted at nematodes than a biocidal fumigant is
- Granular nematicides are not phytotoxic
- They can be applied by growers (do not require a licensed contractor)
- There is a choice of products

- They are incorporated directly into the soil, so can be used in a wider range of weather conditions than spray applied pesticides, with reduced exposure of operators and the above ground environment to the chemical
- They are relatively easy to apply with the correct equipment
- Research into improved application and incorporation of granules has greatly increased their efficacy
- Broadly speaking, supermarkets consider them a more acceptable method of controlling PCN than soil fumigants

Disadvantages

- They are powerful acetylcholinesterase inhibitors and must be treated with care
- There is growing pressure from supermarkets to reduce their use
- If left on the soil surface, the granules pose a threat to birds as they can be mistaken for seed or grit
- They are expensive and there is a strong temptation not to use them when only low levels of PCN are found, even knowing that this could lead to a dramatic increase in the PCN population after harvest
- Their efficacy is greatly influenced by various edaphic factors, such as soil type and their rate of degradation by the soil microbial community
- The persistence of the active material in the soil is variable and, although sufficient to be effective against the quicker hatching populations of *G. rostochiensis*, is less so against the more prolonged hatching of *G. pallida* populations

In May 2003, the decision was taken not to include aldicarb in the Annex 1 list. Although essential use derogation will allow the continued use of the product until 2007, options after this date will be limited. Supermarkets are unwilling to support products for use on food crops where no Annex 1 listing is available.

Only 1,3-dichloropropene is currently available as a chemical fumigant for the control of PCN on a field scale.

8.3 The advantages and disadvantages of soil fumigants

Advantages

- As 1,3-D is a soil sterilant, it has the potential to reduce PCN infestation to zero
- In optimum conditions and applied correctly, reductions of PCN levels by 90% or more have been observed in the upper soil profiles when 1,3-D is used
- An increase in the percentage of marketable yield has been shown in replicated trials when 1,3-D is used, due to a reduction in ‘out’ sizes
- An overall increase in yield due to nitrification has been observed consistently
- Application can be in either spring or autumn and, allowing for weather and soil conditions, the window for application is relatively flexible
- There are no detectable residues in potatoes

Disadvantages

- Soil temperatures must be above 5^{o}C
- Soil moisture should be sufficient to allow a good seal on the surface for that soil type. Organic soils can be difficult to seal sufficiently well enough to prevent the fumigant from escaping
- A contractor is required to apply 1,3-D; conflicting priorities may lead to the fumigant being applied in less than optimum conditions
- 1,3-D has been found to be a direct acting mutagen and a probable human carcinogen
- In certain situations, such as sandy soils with a high water table, 1,3-D can leach into the groundwater
- If soils are too wet, volatilisation and hence penetration through the soil are affected
- If applied in the spring, a break of four weeks is necessary prior to planting potatoes
- In cold, wet, organic, or acidic soils, only nitrate-based fertilisers should be used following the application of 1,3-D, to avoid ammonium toxicity or nitrate starvation
- 1,3-D does not persist in the soil and there is no residual effect, which means that, at depths in the soil profile below that reached by the fumigant, PCN may remain viable and able to re-infest the soil above.

8.4 Mode of action of nematicides

In principle, there are four occasions when nematicides can target PCN during its life cycle: (i) dormant eggs can be killed in the cyst; (ii) hatching of the juveniles can be inhibited, as can movement and location of the roots by the hatched juveniles; (iii) if the chemical action is sufficiently toxic, the juveniles can also be killed directly when moving between the cyst and the root in the soil; (iv) nematicides that are systemic in the plant can have direct toxic effects on those nematodes that have successfully invaded plants.

The mode of action of nematicides is complex and, relative to other areas of nematicide research, there are more published reports on the action of acetyl-cholinesterase (AChE) inhibitors, the primary mode of action of organophosphate and carbamate nematicides. Presumably, there are also data and research results from commercial research, which are not readily available.

In broad terms, there are three steps to investigating the nematicidal properties and potential efficacy of a chemical compound. For example, in the case of AChE inhibitors, making comparative measurements of the ligand recognition sites between different compounds is straightforward when receptor binding assays are used [1]. However, these can be misleading, as Nordmeyer 1992 [2] showed when comparing carbofuran with fenamiphos. The former had a 20,000-fold greater potency than the latter when using these assays but fenamiphos had a greater efficacy than carbofuran against root-knot nematodes in laboratory studies, probably due to the greater stability

of fenamiphos in the nematode. In addition, its first breakdown product is a more potent nematicide than the parent compound.

The fumigant 1,3-D is a biocide and is non-specific in its action. Depending on duration and level of exposure, it has the potential to sterilise soil at the recommended field application rate.

8.5 Edaphic factors affecting efficacy of granular nematicides

All of the physical characteristics that describe a soil affect the efficacy of a nematicide to a greater or lesser degree and none, with the possible exception of pH values, can be treated in isolation. There are two main concerns when selecting a nematicidal compound for use in soil. The first is the rate at which it could be leached from different soils, reducing its efficacy as a control measure and increasing the risk of groundwater contamination. The second is the lipophilicity of a compound, because the greater the lipophilicity the more a compound will be adsorbed onto the organic matter in a soil. Organophosphates are lipophilic in nature and oximecarbamates are hydrophilic. Thus, organophosphates can be affected by the level of organic matter in a soil but pH is considered far less of a problem [3]. On the other hand, oximecarbamates may be affected by high (>8.0) soil pH, such as in the silty loams found around the Wash area, but are considered relatively effective in peaty soils. The rate of chemico-physical degradation of a nematicide in a soil is highly dependent on soil temperature [4, 5], with the time to reach LD 50 greatly reduced at higher temperatures.

Much research has considered the different factors in isolation and in combination, with the resultant data accessible in the public domain. However, due to the complexity of field soils and the highly specific nature of the findings, only broad conclusions are possible. In addition, variation in weather conditions can have a significant influence on the activity and half-life of a nematicide [4], as can the macro structure of a soil. Harvey & Han, (1978) [6] also found that, when investigating the fate of oxamyl in soil, laboratory studies were not confirmed by field studies. For example, oxamyl was found to have a moderate to high mobility in some soils that was not evident in subsequent field studies of the same soil type. The conclusion in this case was that rapid dissipation of the oxamyl in the soil environment minimised any leaching.

Numerous models to simulate pesticide movement and activity in soils have been devised as decision support systems [7]. There are also databases accessible from the internet, such as PETE [8], that give information on chemical compounds with prediction capabilities for environmental behaviour. The models are based on the two main aspects of leaching and persistence, related to the chemo-physical properties of the soil that affect the movement, adsorption and degradation of the active chemicals. Pesticide movement is influenced by factors such as the water balance, adsorption, evapo-transpiration, root uptake and hydraulic conductivity. Simulation of horizontal movement is often given a low priority in these models but this is an important aspect when considering the distribution of nematicide granules in a potato bed applied to control a motile pest, particularly as the effects of the active ingredient may only be transitory, causing only disorientation and not paralysis of movement. Future

research should consider the combination of pesticide movement models with models that describe nematode movement and behaviour in soils.

Although various models, such as the Pesticide Leaching Model [9] and MACRO-DB [8], address pesticide movement in macroporous soils, there would be considerable additional benefit of considering in these models the effects of different soil cultivation techniques and the addition of both organic and non-organic amendments to promote persistence of the nematicides in the rhizosphere of the potato crop. A greater volume of soil is managed for a potato crop than for any other arable crop and there is consequently greater scope for manipulating the macro structure of the soil through different tillage techniques. The formation of a false 'pan' at an optimum depth might be one such approach.

8.6 Biodegradation of nematicides

Microbial degradation is an important factor in the efficacy of nematicides but is rarely considered by growers and agronomists when addressing evidence of poor control by a nematicide in a field situation. There is considerable evidence to show that repeated use of a single nematicide can lead to increased breakdown or transformation of that compound at each subsequent application [10, 11, 12]. The phenomenon of cross-adaption has also been demonstrated between pesticides of a similar family [10, 13], such as when soils repeatedly exposed to either aldicarb or oxamyl, and showing increased rates of degradation, had a similar effect on the other compound. Soil pH appears to have an effect on microbe communities that degrade nematicides, with accelerated rates found in a soil of pH 7.3 but not in a soil of pH 5.6. [13], suggesting an avenue for further investigation and development.

8.7 Commercial use of nematicides

A grower can make one of two key decisions when PCN is found in a field targeted for potato production. The first is to look for alternative, PCN-free land, which is an economic and logistic decision. The second is whether or not to apply a nematicide to the field. In both cases, the decision tends to be short-term and tactical rather than strategic, although this is slowly changing as growers become more accustomed to treating PCN management as a long-term rather than a seasonal problem. There is a limited amount of PCN-free land capable of growing a profitable crop of potatoes that is not already taken up. Also, landlords are increasingly requiring growers to leave fields with post-harvest levels of PCN similar to pre-cropping levels, thus forcing the tenants to use nematicides, especially at low levels.

Currently, the accepted recommendation from independent advisors is to treat a field with a granular nematicide if any PCN is found and to target areas and fields above thresholds varying from 15 to 20 eggs/g of soil with a fumigant such as Telone. If treatment is not applied, there is a risk of crop loss in the immediate crop, although the amount of damage is dependent on other factors, such as the resistance and tolerance of the potato cultivar grown. However, in PCN, the potential for reproduction is very great (as many as 500 eggs from each female) and this can leave a serious problem for following potato crops. Even with this knowledge, some growers may choose not to apply a nematicide to reduce costs for that crop. Others may choose only to apply a fumigant, even in the knowledge that efficacy is never

100% and that the surviving nematodes will multiply and leave a problem for the following crop.

8.8 Research into nematicides and their use for the control of PCN

In the last ten years, only two main research projects involving nematicides have attracted some degree of public funding in the UK. An important part of both projects has been the population dynamics and distribution of PCN at the field scale. Alternatives to chemical nematicides attract greater interest from public funding. This is a commendable trend but ignores the continuing necessity for the potato industry to use nematicides for the foreseeable future. With increasing pressure from supermarkets and consumer groups to reduce nematicide use, the industry, including representatives of the agro-chemical sector, is looking at ways of increasing the efficacy of nematicides as well as looking at possible alternatives. The industry as a whole is wary of the promise of alternatives as, in the past, some have been put forward as sweeping panaceas for the control of PCN but have not been as effective or ready to use as indicated. The future will see a more gradual change, with alternative and traditional management techniques working in conjunction rather than isolation.

8.9 Research and the agro-chemical companies

Overall, much of the research into nematicides, in particular their behaviour in soils, has been undertaken by the chemical companies themselves. This has been firstly as preparation before launch, and then to respond to feedback from commercial use. The results and conclusions are often viewed as commercially sensitive and access can be very limited. However, with the advent of the Defra LINK programme, there is greater exchange of information and more open dialogue between the chemical producers, the rest of the industry and academia.

Apart from the fumigant 1,3-D, the chemical nematicides now available in the UK for PCN control, were developed in the 1960s, 70s and 80s, the most recent being fosthiazate (Nemathorin). If we accept that nematicides are necessary for economic production of potatoes which, along with sugarbeet, is the main cash crop in many areas, the development of a new nematicide, using up-to-date methods of development and screening, is a viable prospect. Nematicides are expensive on a per hectare basis compared to foliar insecticides and it was recently estimated that the potential market for Temik (aldicarb) was in excess of £5M annually in the UK alone; this is apparently sufficient to justify investment in a new product (M. Tate, pers. comm.). The advantages to the rest of the industry and to consumers of produce protected by a new pesticide could be many. There is potential to improve efficacy and reduce active ingredient loading, to improve targeting of the chemical, and to have a product more acceptable to consumers. If a nematicide could be safely applied as a foliar spray and then translocated by the plant to the roots, the targeting would be greatly improved and the considerable problems presented by edaphic factors and the distribution of the granules would be avoided. However, such a mode of action would only be acceptable in a nematicide used for long-term, strategic management of PCN. Allowing the nematode to invade the host plant before killing it would mean accepting the damage caused, and this would be tolerable only with a very low PCN population that was under effective management.

Granular nematicides are incorporated into soil before seed potatoes are planted. For them to remain biologically active over the hatching period of PCN, large amounts of active substance have to be applied. Drip irrigation systems provide an effective means of delivering measured doses over the key time period, potentially leading to use of lower overall levels of active ingredient (a.i.) [14]. The efficacy of this approach has already been successfully demonstrated in glasshouse pot trials using levels of a.i. reduced by more than 30%. The increased pressure to reduce water usage makes trickle irrigation systems attractive and, with their potential for patch application of nematicides to hotspots of infestation, they may also offer a workable system for reducing nematicide load in the environment. This is particularly the case where fumigants are not useable due to high levels of organic matter, or where soils have a high content of stones. Both types of soil prevent proper sealing and so allow rapid loss of active ingredient from the soil.

8.10 Application and incorporation of granular nematicides

This is an area of research and development that has probably seen the greatest level of collaboration, chiefly between the agrochemical companies themselves and research institutes. Aspects such as the formulation of the granules to aid incorporation, the influence of different soil types and the cultivation methods used must be considered collectively. Further research and development is necessary, particularly looking at the safe handling and field use of granules with the aim of reducing exposure risk.

8.11 Recommendations

- *Develop trickle irrigation for the improved application of nematicides*
- *Combine information on nematode and pesticide movement in soils in models with reference to PCN on potatoes*
- *Identify key species of soil microflora involved in the biodegradation of nematicides in soils, and develop markers and strategies for their management*
- *Undertake studies on the behaviour and activity of fosthiazate in different soil types and under varying environmental conditions*

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9. MODELLING AND SAMPLING OF PCN

9.1 Modelling PCN

The population dynamics of PCN at the field level depends on numerous complex and interacting factors that have to be included when developing a model [1]. For example, such elements as susceptibility and tolerance to PCN attack of the potato cultivar, efficacy of nematicides, genetic background of the population and decline rate must all be integrated. Although a number of models have been constructed for various aspects of PCN dynamics, a recently developed predictive model seeks to incorporate the majority of relevant factors that may influence a field population of PCN (part of SA-LINK project 112). This model, though complex, has been constructed to facilitate its use with the minimum of introduction or training and placed on a CD-ROM for easy distribution. The model this is a tool for education and management purposes and represents the current state of the art in this area. However, two important considerations remain: first, there is an urgent need for more empirical data for most of the aspects such as cultivar and soil type, to support some of the suppositions and improve the robustness of the predictions; second, although the model is functional, further work is required to improve the programming, increasing flexibility of use but at the same time removing any potential for misleading prediction.

9.2 Recommendation:

- *It is strongly recommended that the model is developed to a level robust enough for general use both in industry and research community.*

9.3 Sampling for PCN

Sampling for PCN at a field scale is complex, due to the patchy distribution of the pest both horizontally across the field and vertically through the soil profile. Although many publications address the problem [2-15], there are still no definitive methods other than the now somewhat outdated EPPO guidelines. However, it has been demonstrated that intensive sampling of a high value crop like potatoes can be justified and the risks calculated even for individual points sampled and processed at spacings as low as 20 m [16].

There is an urgent need to combine the thinking from previous studies and to develop that is both theoretically sound and based on real data. Such a programme of studies would yield a series of risk analyses that both industry and research community could use.

9.4 Recommendation:

- *A two-year research programme into sampling and methods of estimating PCN populations at field scale for both the industry and research community. The work should include the input of a modeller and an economist.*

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10. ALTERNATIVE CONTROL MEASURES

10.1 Introduction

The more traditional methods for controlling PCN are those involving nematicides, resistant cultivars and crop rotation. However, there is a continuing search for alternatives that may either replace or be used in conjunction with established methods. In the past, some of the methods termed 'novel' have not been considered seriously. Nowadays, due to the pressure from supermarket protocols and the threat of a pesticide tax, various approaches are being reconsidered by the industry helped, in some cases, by technological advances that make them more practical.

Physical control methods as alternatives to nematicides, such as the use of electromagnetic fields (EMF), have the distinct benefit of potentially being able to control a wide range of nematode species. They may also not require any specialised knowledge by the grower, as the operations would almost certainly be performed by a contractor. These methods are highly suited to integrated management approaches as the application is instant and leaves no residues, although the risk of phytotoxic breakdown products is a consideration. There may also be additional benefits, such as making PCN cysts that do not receive lethal doses more susceptible to biological control agents due to increased degradation of the egg shell membrane.

With the imminent advent of 'de-coupling', there is potential for greater use of cover crops by the industry, particularly as a replacement for a cereal crop in a rotation and where potato growing land is at a premium. The use of trap cropping with potatoes, or with a fully resistant trap crop such as *Solanum sisymbriifolium*, would then be more attractive on a field scale rather than just for patches of high PCN infestation.

The greatest difficulty with novel methods of control is the funding of the interim research between 'proof of principle' and the developmental research leading to industry uptake. In the case of EMF, this could be expensive in terms of the development of technical equipment compared to other, more biologically-based studies.

Many alternative control methods and strategies have been studied and it is not within the scope of this document to detail them all but to highlight those which have recently generated greatest interest in the industry and which there is a scarcity of knowledge in the agricultural sector.

10.2 Cover crops

There are a number of potential mechanisms through which 'cover crops' can control plant parasitic nematodes. The most widely investigated is one in which the life cycle of the pest is interrupted to such an extent that numbers are greatly reduced, generally referred to as trap cropping. Trapping out a nematode pest can be achieved using either a sacrificial, cash crop or a plant species that has no intrinsic economic value other than for this sole purpose. Other systems rely on the production of toxic exudates by the growing crop or where there are breakdown products from the crop that are toxic to nematodes when the cover crop is incorporated into the soil.

10.2.1 Trap cropping PCN with potatoes

This is a method that has generated considerable interest from growers and researchers almost since PCN became recognised as a problem for the economic production of potatoes. Two early papers from the 1930s considered and tested the practical value of such an approach [1, 2]. Since then, there have been many investigations, with effective trap cropping reducing PCN populations by as much as 87% [3] or more [4]. However, trap cropping is not always as effective as hoped and some field-based studies have resulted in reductions of 40% or less (Peter Cornish, pers. comm.). The cost of establishing a commercial potato crop can be more than £3,500/ha and the possible economies that might be made to reduce this cost when planting a potato trap crop need to be substantial before the procedure can be justified. The cost of fumigantion is £600/ha and this could be seen as the target cost, athough the use of a fumigant or trap crop are management options mainly used to reduce PCN populations to a level that is controllable with a granular nematicide applied at planting. With this in mind, the cost of a trap crop should be considered in more strategic terms than just in the context of the next potato crop, particularly where an organic textured soil may preclude the use of a fumigant. Certainly, this is a method that could be adapted for organic production. There is increasing pressure from consumers, and therefore supermarkets, to reduce overall usage of pesticides and, in particular nematicides. Supermarket protocols are important controlling mechanisms in this objective.

Trap cropping has many benefits as an alternative management method, perhaps the most important being its flexibility of use within the growing season and its relatively short duration of between five and six weeks, depending on soil temperature. Recent studies undertaken as part of SA-LINK project 112 demonstrated that trap cropping can be successfully used in the late summer and autumn as well as during the more traditional spring planting period. It is also possible to produce a worthwhile yield from a trap crop for such markets as canning [4, 5].

The efficacy of a trap crop is dependent on the length of time that the crop can be left in the ground and so trigger as many of the PCN to hatch as possible, without allowing the individuals that hatched first to complete their life cycle. Activity and physiological development of PCN only occurs above basal temperatures, which for *Globodera pallida* is 3.9^{o}C and for *G. rostochiensis* is 6.2^{o}C. Accumulated day degrees (DDC) above these thresholds are used to estimate what stage of development the PCN have reached and, therefore, when the crop should be lifted.

In summary:

Advantages

- There is a potential to reduce *G. pallida* by 75% to 80%.
- If glyphosate is not used to destroy the crop it can be acceptable for organic production.
- With careful timing and management it may be possible to produce an economically viable extra potato crop.
- The principle and the agronomy used are methods that are understood by the industry and only traditional potato cropping machinery is required.
- There is a relatively short operation time of between five and six weeks.
- It is a method that can be used throughout the growing season.

Disadvantages

- It is a method with a high compared to most other pest control strategies.
- The destruction of crop is weather dependent, particularly when glyphosate is not used and the crop has to be removed to prevent PCN multiplication.
- The overall efficacy of a potato trap crop is dependent on the majority of the PCN population involved, hatching within the target accumulated DDC. A slow or late hatching population may not be trapped sufficiently well to justify the costs.
- Some populations of PCN have been shown to have different basal temperatures for activity.
- The method can be difficult to incorporate into farm rotations.
- The approach requires careful management that initially, may require expert advice at key times.
- In many situations, trap cropping will depend on glyphosate application being fully effective.
- Soil temperatures need to be monitored accurately and regularly.

Although there is published information on individual aspects of trap cropping with potatoes, there are no clear guidelines or even workable parameters such as defined DDC or plant spacing for a range of situations. A single project is required to bring all the aspects together and some should be addressed more comprehensively.

Consistency in reduction of PCN populations requires:

- Further work on promoting rate and spread of root growth
- Further studies into differences in temperature thresholds of development between different populations of PCN
- The establishment of optimum parameters for different planting times, soil types, PCN populations and management situations
- The development of a user friendly system for monitoring DDC

Trap cropping with potatoes is a potentially useful and effective tool that requires, but justifies, further research.

10.2.2 Trap cropping with Solanum sisymbriifolium

Trap cropping PCN with a non-tuber-forming *Solanum* species is an attractive approach as the crop is destroyed more effectively with a herbicide or through cultivation, than a crop grown from tuber seed. Plants grown from tubers can recover and re-grow unless the tuber is removed, a relatively expensive task in terms of labour and machine use. A number of plant species grown from seed are known to trigger hatch of PCN but, although invasion of the roots may occur, PCN is unable to complete its life cycle [6]. *Solanum sisymbriifolium* is an example of a species in which hatch is triggered and root invasion occurs but rarely are any later life stages found to indicate that further development occurs.

The advantages and disadvantages of *S. sisymbriifolium* for trap cropping PCN in the UK:

Advantages

- Fully resistant to PCN
- Triggers hatch
- Easy to destroy
- Resistant to selected herbicides, so chemical weed control is possible
- Frost tolerant
- Blight resistant
- Can reduce PCN by more than 75%

Disadvantages

- Slow to establish
- Prefers low pH
- Reduced growth in sandy loams
- May require irrigation to establish
- Requires fertiliser application and weed control
- General agronomic management requires further research
- Efficacy is variable depending on PCN population
- High thermal activity threshold of seed lines currently available

Although *S. sisymbriifolium* has the potential to be an effective and manageable trap crop for PCN control and research continues in The Netherlands (Van Dijke Semo, pers. comm.), a number of agronomic issues directly pertinent to the UK require research and clarification before the crop should be used on commercial scale, including establishment and weed control. In addition, there is a marked inconsistency in effect of *S. sisymbriifolium* on different populations of PCN (SA-LINK 112). Further work is necessary, perhaps using new cultivars of this plant species.

10.2.3 Soil amendments

The incorporation into soil of some crops such as *Brassica* spp., can release large amounts of glucosinolates into the soil, and these form isothiocyanates when hydrolyzed by the enzyme myrosinase. *In vitro* studies have shown 100% mortality of *G. rostochiensis* occurring after eight hours of exposure to 2-phenylethyl glucosinolate and myrosinase [7]. Other plants, such as *Tagetes* spp. have also been investigated, particularly in South America where the incorporation of leaves and flowers of *Tagetes minuta* were found to decrease reproduction of a PCN population by 72%.

The use of cover crops to produce nematicides has obvious attractions for the organic grower but increasingly, growers using conventional methods are also contemplating this as an option, particularly when de-coupling and low cereal prices are considered. There are also commercially available preparations of plant or fungal origin that have some nematicidal action such as garlic and DiTera™, both of which require further research to establish their value as methods for controlling PCN at field scale.

The use of biofumigants and other biological compounds could be of considerable value but has only received fragmented attention. Until clear guidelines with potential to yield consistent results are forthcoming, they are unlikely to be seen as an option by the majority of potato growers.

10.3 Chemicals affecting hatch

There are two categories of agents that effect PCN hatch, those that stimulate hatch and would be used in the absence of a commercial potato crop, and those that inhibit hatch. Research has looked at both artificial and plant-derived agents, with some results indicating that the approach has some prospect. However, there are production and storage issues for use at field scale [9].

Perhaps the most important recent advance has been the identification and characterisation of solanoeclepin A, a hatching agent produced by potato plants (10). After processing, 0.245 mg were isolated from 1,000 potato plants. Some preliminary work suggests that only 0.3 g/ha are required to affect PCN. Although the synthesis of the compound is proving to be difficult, there may be other approaches in which solanoeclepin can be exploited, such as its production from a GM cover crop. A non-food crop such as *Solanum sisymbriifolium* with enhanced expression of solanoeclepin would potentially be a very useful option.

10.4 Physical controls

10.4.1 High frequency electrical fields (HFEF)

From as far back as the 1880s, there has been ongoing interest in the use of electrical fields in agriculture for applications such as the control of weeds in crops [10] and, for a period at the beginning of the 20th century, their beneficial effects on crop growth [11]. Early research into the use of HFEF to control pests of seeds showed that fungi such as *Fusarium* spp. could be killed with only a 10% reduction in germination of the wheat seed which it was infecting [12] and that 36 litres of wheat exposed to a 0.27 kWh HFEF would be free of grain weevil. These effects were considered to be due to dielectric heating but in the 1950's and 1960's, non-thermal effects were indicated [13] and research into the use of pulsed electrical fields to kill bacteria, particularly in food, demonstrated the selective damage of inner cell membranes [14, 15].

The use of HFEF for the control of soil-borne pests such as nematodes has proven more difficult to clarify, particularly when discriminating between thermal and non-thermal effects. Currently, the power requirements to rapidly heat a volume of soil in a field situation to more than 50^{o}C (the nominal temperature for killing *G. pallida*), is prohibitive. In the 1950s, a series of academic publications gave results and conclusions conflicting in nature from investigations conducted in Hawaii, Zimbabwe and the USA at both laboratory and field scale [16-18]. However, the possibility of achieving PCN control through the non-thermal effects of HFEF would be of practical application.

A recent, industry-commissioned, preliminary study undertaken at Rothamsted Research indicated the feasibility of HFEF as a method to control PCN in a field situation [19, 20]. Further work at glasshouse scale looking at variables such as soil

type and moisture content, is required before a more thorough assessment can be made of the method as a practical means for PCN control at field scale. However, an appraisal made by a specialist company suggested that the engineering of a device for agricultural use was well within the limitations of current technology (David Terry, Castlet Ltd, pers. comm) [21].

A key, non-thermal effect, observed for both PCN and *Meliodogyne incognita* [19, 22] but not for *Heterodera schachtii,* is the induction of hatch when eggss are exposed to certain HFEFs. Stimulation of hatch would be suicidal without the presence of a potato crop and therefore very appropriate as a means of control. This phenomenon is almost certainly due to the disruption of the egg shell membrane, altering its permeability and allowing the trehalose that maintains the egg in diapause to diffuse out, and the juvenile to rehydrate and hatch. Although power requirements for this effect appear relatively low, the field characteristics may be quite specific making replication in a heterogeneous soil impractical. However, a rapidly changing series of electrical fields, designed to cover a range of alternatives, could be applied to achieve the same overall effect.

10.4.2 Microwaves

Within the electromagnetic spectrum, radiation at a frequency of between 3 x 10^{11}-10^{13} Hz and a wave length of 1mm - 30cm is described as microwaves. Through international agreement, 2450 Megahertz (MHz) is the frequency allocated for domestic and industrial microwave use. During the 1970s, there was increased research into the use of microwaves for controlling weeds as a replacement for herbicides, through killing the growing seedlings and the seeds in soil [23-26]. Although results were promising, considerable variation in efficacy was found between soil types, which was probably due to attenuation of thermal effects by variation in soil moisture levels. In 1971, the Phytox corporation built a prototype machine for controlling weeds with microwaves capable of delivering 60 kW with an estimated daily work rate of between 0.4-2ha. Although the machine was effective, it weighed over 33 tonnes, was expensive to construct and safety was a major concern.
Other work in the 1970s and 1980s looked at the use of microwaves for the control of soil-borne pests such as fungi and nematodes, and relied on the thermal effects of the energy input. The results were variable because, as soil moisture increased, penetration into the soil was reduced [27]. The conclusion by many authors was that the application of microwaves for the control of nematodes and fungi in soil would require marked improvements in the equipment before an agricultural scale applicator could be produced [28-30].

A characteristic of microwave application is the selective heating of water in a heterogeneous material such as soil, which would greatly reduce the energy required if the water were the target. Hydrated weed seeds were found to be more susceptible to microwave radiation than non-hydrated seeds [25, 26]. Free-living nematodes and juveniles of the cyst-forming species inhabit the water layer that surrounds soil particles. In addition, cysts themselves contain water, suggesting that the microwave energy would be relatively targeted and more ecologically benign than fumigation with a biocide.

Recent studies showed that with pre-soaked cysts of PCN exposed to 25 seconds of microwave radiation in a 1.2kW oven there was a four-fold reduction in percentage hatch [31]. For field-scale applications, it is probably not practical to use such a duration of exposure but it does indicate the efficacy of microwaves against PCN. More recent preliminary studies undertaken as part of an on-going SA-Link project, (Integrated Management Strategies for the Control of PCN) showed that there was a six-fold reduction in hatch of *Globodera pallida* from cysts in a sandy loam soil with a 10% moisture content soil exposed to 10 seconds of radiation in a 650W oven. There was only an 8^{o}C rise in temperature (from 15 to 23^{o}C) suggesting that there was a targeted effect of the energy. Whether or not this effect is thermal in nature is unclear and requires further investigation. However, with the considerable advances in technology and reduced cost of equipment such as domestic and industrial size magnetrons, this approach has the potential to be a cost effective method for the non-chemical control of PCN. In particular, the lifting of the soil in which the potato crop will be grown, during such operations as de-stoning, means that the microwave applicator can be designed to treat just the soil on a conveyor belt or elevator for example, which would require treatment of a much reduced volume of soil than treating field soil *in situ*. A microwave device is ideally suited to treat soil from the grader before the soil is returned to the field, would greatly reduce the level and spread of PCN at field and farm scale.

10.4.3 Steam sterilisation

Steam sterilisation on an agricultural scale is technically feasible and there are machines operating on a commercial basis in the UK and Europe that sterilise soil as they move across a field, as opposed to using plastic sheets in a semi-immobile process [32-36]. However, the machines are very slow and may only cover two hectares a day in the most optimum soil conditions. The cost can also be hard to justify for the control of PCN alone, reaching £2,500/ha for up to 25 cm depth of soil treated. The effects of steam and associated temperature rise on PCN in soils is sufficiently well understood that the problem is more one of technical development of the machines than research into the effects of steam on the nematode.

10.4.4 Other physical control methods

Solarisation of soils for the control of nematodes is a method not usually considered relevant in temperate climates as extended periods of strong sunlight are necessary. However, with increasing evidence of climate change, the effects of warmer soils during the summer should be taken into account when assessing decline of PCN between potato crops in the UK. Manipulation of this effect through the application of plastic sheeting is not practical, nor cost-effective on an arable field scale. But, leaving fields cultivated and without a crop cover during periods when temperatures are high may reduce PCN levels in the top few centimetres of soil, and repeated cultivation will increase the effect.

While there are other physical methods for controlling PCN, such as infrared and gamma irradiation, they are limited in their application either through safety issues or the need for further, considerable technical development, with no guarantee of success. Flooding as a means for controlling PCN has recently been demonstrated to be effective [37], but the situations where it might be applicable are very limited.

10.6 Recommendations

To some degree, non-conventional methods of PCN control are subject to far greater levels of scrutiny and criticism from the industry than a new chemical control. In itself, this is commendable but difficulties of moving from glasshouse or small plot to field-scale can be a major hurdle requiring capital investment. Field scale trials are necessary to highlight unforeseen difficulties such as effects of soil type or structure, and this may lead to further laboratory research before a near-market system or method is developed. As these methods may be novel in their approach, the effects on non-target organisms should always form part of any research.

Recommendations for further research:

- *Trap cropping with potatoes - assimilation of research to date and the development of a series of definitive protocols and recommendations for field scale, commercial use.*
- *Trap cropping with* Solanum sisymbriifolium - *improved understanding of the agronomy and manipulation off the crop to promote control of PCN, further studies looking at the efficacy of the crop against different populations of PCN; testing of new seed lines.*
- *Further studies into biofumigants for the control of PCN and other nematodes with the possible additional benefits of green manuring plus investigation of effects of biofumigants on non-target organisms.*
- *Development of microwaves for the targeted control of PCN and other crop pests using non-thermal, specific effects.*
- *Development of HFEF using non-thermal effects for the targeted control of PCN and other plant parasitic nematodes.*

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11. APPENDICES

The following recommendations are made in the understanding that certain basic requirements will be fulfilled. These include the maintenance of germplasm collections to underpin future breeding work, and the recognition that some increase in funding for research into the biology and control of *Globodera pallida* is essential for the continued health of the British potato industry. An appropriate way forward for this would be for Defra to establish a research and development committee to coordinate the longer term objectives and funding for research on PCN, with representatives from other funding agencies, research teams and industry. Added benefit from such a coordinated approach could be obtained if research projects were also to include work on other UK plant parasitic nematodes where appropriate. Finance should also come from more than one agency to support a UK consortium of molecular nematologists to construct a physical map of the genome of *G. pallida*, in collaboration with a centre able to provide genomics and bioinformatics support. The resultant map should be placed in the public domain to maximise its exploitation for gene discovery. Such collaboration would help draw the UK nematology community together and provide information essential to the exploitation of transgenic crops - exploitation that will eventually come once the technology is developed in a manner in which it becomes acceptable to the public. At this stage, work to produce potato cultivars with engineered resistance to PCN will assume a higher priority.

11.1 Recommendations

5 year recommendations

1. Increase efforts to breed commercially acceptable *G. pallida* resistant cultivars by conventional breeding techniques.
2. Evaluate and develop the SA-LINK PCN population dynamics model as a management tool.
3. Develop trickle irrigation for the improved application of nematicides.
4. Compare potatoes and different lines of *Solanum sisymbriifolium* as trap crops.
5. Assess whether selected bacteria and fungi can be increased in the rhizospheres of PCN non-host crops and thereby increase rates of decline.
6. A two-year research programme into sampling and methods of estimating PCN populations at field scale for both the industry and research community. The work should include the input of a modeller and an economist.
7. The GM potato lines expressing proteinase inhibitors should be tested more widely, both in containment and under field conditions, to evaluate their durability against different PCN populations and the transgenes transferred to commercially acceptable cultivars.
8. Assess whether sustainable control of PCN is best achieved through the manipulation of the residual microflora in potato land or through the application of selected agents.
9. Identify molecular markers for virulence and develop rapid quantitative diagnostic tests.

10. Identify key species of soil microflora involved in the biodegradation of nematicides in soils, and develop markers and strategies for their management
11. Develop understanding of the population genetics of PCN populations within fields and its impact on the deployment of resistant cultivars.
12. Combine information on nematode and pesticide movement in soils in models with reference to PCN on potatoes.
13. Undertake studies on the behaviour and activity of fosthiazate in different soil types and under varying environmental conditions.
14. Development of microwaves for the targeted control of PCN and other crop pests using non-thermal, specific effects.
15. Determine whether selected microbial agents or non-host cultivars can increase decline rates of PCN between potato crops and whether microbial agents slow rates of selection of virulent populations on partially resistant cultivars.
16. Development of HFEF using non-thermal effects for the targeted control of PCN and other plant parasitic nematodes.

5-10 year recommendations

1. Use molecular techniques to identify, characterise and possibly modify new sources of natural resistance genes against *G. pallida*.
2. Through the development of public-private partnerships develop selected isolates of fungi or bacteria as biological control agents against PCN and related cyst nematodes.
3. Identify, characterise and inhibit the activity of genes involved in important aspects of the life cycle of PCN, which may be exploited through transgenes, new nematicides or semiochemicals. This will provide additional novel resistance genes should the proteinase inhibitors not prove durable and new bioactive compounds should GM crops remain unacceptable in Europe.
4. By studying the infection processes and antagonism of selected agents identify and characterise bioactive compounds for use as novel nematicides or sources of plant resistance.
5. Identify factors that influence the deposition of lipids in second-stage juveniles and attempt to minimise the lipid content of nematodes to reduce their long term survival and infectivity.

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11.3 List of presentations: Open forum to discuss PCN research priorities

Session 1: ***The demands of the market place***

1) Guy Gagen (BPC). The potato crop and its economics
2) Tony Worth (QV Foods). The grower's perspective
3) David Nelson (Branston). The viewpoint of packers and suppliers
4) Peter Harkett (McCain). The viewpoint of potato processors
5) Sandy Norman (Tesco). The supermarket view
6) David Richardson (PSD). Regulation and nematicides

Session 2: ***Current PCN management options***

1) Pat Haydock (Harper Adams). The current PCN situation
2) Jon Pickup (SASA). The EU PCN directive
3) Ken Evans (R-Res). Precision management tools
4) Tudor Dawkins (Du Pont). Nematicides
5) Paul Gans (NIAB). Resistant cultivars
6) Sue Hockland (CSL). Options for the organic grower

Session 3: ***Research towards future management options***

1) Glenn Bryan (SCRI). PCN variation and breeding for resistance
2) Brian Kerry (R-Res). Biological control using microbial agents
3) Andy Barker (R-Res). Novel approaches to control
4) David Bird (NCSU). Gene discovery for PCN control: a models approach
5) Charles Opperman (NCSU). Exploiting nematode genomics
6) Howard Atkinson (University of Leeds). Transgenic nematode resistance
7) John Pickett (R-Res). Signalling in the rhizosphere

Session 4: ***Technology Transfer and R & D needs***

1) Rob Clayton (BPC). Improvements that can be made by exploiting current technology and improved routes of technology transfer
2) Mark Phillips (SCRI). Sampling and modelling
3) Andy Barker (R-RES). SA-LINK
4) Bill Parker (ADAS). Final discussion